09/544910

Examiner: Rawlings, S.
Art Unit: 1642

(FILE 'HOME' ENTERED AT 17:27:59 ON 21 APR 2002)

FILE 'MEDLINE, CANCERLIT' ENTERED AT 17:28:20 ON 21 APR 2002

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L1           70 S ANTISENSE (10A) (LIMITATIONS OR FAILURE OR
(LACK(2W)(SUCCES?
L2           50 DUPLICATE REM L1 (20 DUPLICATES REMOVED)
L3          417 S ANTISENSE (10A) (CLINI? OR PHASE(3W)TRIAL OR PATIENT)
L4            1 S L3 AND APOE
L5            1 S L3 AND APOLIPOPROTEIN
L6            0 S L3 AND (VLDL OR LDL)
L7            1 S L3 AND (ARTERIO? OR ARTHER?)
L8           95 S ANTISENSE AND (APOE OR APOLIPPROTEIN OR VLDL OR LDL)
L9           26 S ANTISENSE (10A) (APOE OR APOLIPPROTEIN OR VLDL OR LDL)
L10          20 DUPLICATE REM L9 (6 DUPLICATES REMOVED)
L11           1 S L3 AND (ARTERIO? OR ARTHE?)
L12           2 S ANTISENSE AND APOE3
L13           4 S L3 AND (HEART (W)DISEASE OR APOE OR APOLIPOPROTEIN OR
LIPOPRO
L14           4 DUPLICATE REM L13 (0 DUPLICATES REMOVED)
L15           1 S ANTI-ABCA1 ANTISENSE
L16          37 S ANTI-APOE
L17          35 DUPLICATE REM L16 (2 DUPLICATES REMOVED)
L18          31 S (GEMFIBROZIL OR PREVASTATIN) AND ((APOLIPOPROTEIN OR APOE
OR
L19          29 DUPLICATE REM L18 (2 DUPLICATES REMOVED)
L20        7830 S APOLIPOPROTEIN(W)"E" OR APOE OR APOE3 OR APOE-3 OR
APOLIPOPRO
L21         378 S L20 (10A) (GENE(W)EXPRES? OR MRNA OR TRANSCRIPT?)
L22          39 S L21 (10A) (DESCREAS? OR INHIBI? OR REDUC? OR BLOC?)
L23          33 DUPLICATE REM L22 (6 DUPLICATES REMOVED)
L24          34 S INSULIN THERAPY AND (HYPERLIPOPROTEINAEMIA OR
HYPERLIPIDEMIA)
L25           0 S DEXAMETHASONE THERAPY AND (HYPERLIPOPROTEINAEMIA OR
HYPERLIPI
L26           5 S DEXAMETHASONE (10W) (HYPERLIPOPROTEINAEMIA OR
HYPERLIPIDEMIA
L27           5 S ASPIRIN (10W) (HYPERLIPOPROTEINAEMIA OR HYPERLIPIDEMIA OR
HYP
L28           0 S (INHIBITOR(3A)(NUCLEAR FACTOR KAPPA B OR NFKAPPAB)) (10W)
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FILE 'MEDLINE' ENTERED AT 19:45:31 ON 21 APR 2002

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L29           0 S L28
L30           0 S (INHIBITOR(3A)(NUCLEAR FACTOR KAPPA B OR NFKAPPAB)) (10W)
(HY
L31           0 S (INHIBITOR(3A)(NUCLEAR FACTOR KAPPA B OR NFKAPPAB)) (10A)
(HY
L32           0 S (INHIBITOR(3A)(PEROXISOME PROLIFERATOR)) (10A)
(HYPERLIPOPROT
L33           0 S (INHIBITOR(3A)(NSAID)) (10A) (HYPERLIPOPROTEINAEMIA OR
HYPERL
L34           0 S (INHIBITOR(3A)(COX)) (10A) (HYPERLIPOPROTEINAEMIA OR
HYPERLIP
L35           2 S NSAID (10A) (HYPERLIPOPROTEINAEMIA OR HYPERLIPIDEMIA OR
HYPER
L36           9 S DEXAMETHASONE (10A) (HYPERLIPOPROTEINAEMIA OR
HYPERLIPIDEMIA
L37           7 S (PHORBOL OR TPA) (10A) (HYPERLIPOPROTEINAEMIA OR
HYPERLIPIDEM
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L38             0 S (BIOTIN) (10A) (HYPERLIPOPROTEINAEMIA OR HYPERLIPIDEMIA OR
HY
L39             7 S L21 AND (FIBRATE OR FENOFIBRATE OR CHOLESYRAMINE OR
LOVASTATI
L40             2 S (MEVINOLIN) (10A) (HYPERLIPOPROTEINAEMIA OR HYPERLIPIDEMIA
OR

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STIC-ILL

A51,05

From: Rawlings, Stephen
Sent: Sunday, April 21, 2002 8:10 PM
To: STIC-ILL
Subject: ill request

Art Unit / Location: 1642/CM1,8D12

Mail box / Location: Rawlings - AU1642 / CM1, 8E12

Telephone Number: 305-3008

Application Number: ~~09583638~~ ApoE

Please provide copies of the following references:

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Journal code: D0M; 100891485. ISSN: 1464-8431.

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Therapy Reviews

Contemporary recommendations for evaluating and treating hyperlipidemia

RICHARD S. PERRY

Abstract: The biochemistry, etiology, and evaluation of hyperlipidemia and its management, including dietary and drug therapies, are discussed.

Strong evidence supports the role of increased cholesterol concentrations as an independent risk factor for coronary artery disease (CAD); however, evidence that elevated triglyceride concentrations are also an independent risk factor remains questionable. The cornerstone of the laboratory diagnosis of hyperlipidemia involves repeated measurement of serum or plasma cholesterol and triglyceride concentrations.

The goals of therapy should be to reduce cholesterol or triglyceride concentrations or both to below the 75th percentile, modify co-existing risk factors, individualize the treatment, and minimize any adverse effects. Specific interventions must be determined on the basis of patient age, gender, etiology of hyperlipidemia, presence of other risk factors, and degree of lipid abnormality. The majority of patients may be managed with dietary therapy alone. The three-phase diet developed by the American Heart Association emphasizes a gradual reduction in cholesterol and fats with the substitution of polyunsaturated for saturated fats. Patients at risk for CAD with sustained elevations in plasma cholesterol concentrations above the 95th percentile or a triglyceride concentration above 500 mg/dL after an adequate dietary trial should be considered for drug therapy. The effects of cholestyramine and colestipol hydrochloride, niacin, dextrothyroxine, clofibrate, neomycin sulfate, probucol, gemfibrozil, and mevinolin and compactin on lipids and lipoproteins are reviewed.

Hyperlipidemia should be managed systematically using information about the association between increased lipid concentrations and CAD, patient risk factors, and limitations of both diet and drug therapy.

Index terms: Antilipemic agents; Cholestyramine resin; Clofibrate; Colestipol hydrochloride; Compactin; Dextrothyroxine; Gemfibrozil; Hyperlipidemia; Mevinolin; Neomycin sulfate; Niacin; Nutrition; Probucol

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Over the past 15 years extensive evidence has accumulated documenting the importance of elevated serum cholesterol in the pathogenesis of coronary artery disease (CAD).[1-3] Evidence for the role of triglyceride in CAD is far less convincing.[4] Recently, the results of a large multicenter trial have focused attention on the management of elevated serum lipids and generated new recommendations for treatment.[5-9] This paper briefly reviews the biochemistry of lipids and lipoprotein metabolism, etiology of hyperlipidemia, and evidence supporting increased plasma lipid concentrations as risk factors for CAD. The evaluation and management of patients with hyperlipidemia, including the types of therapy and the benefits and limitations of treatment, are discussed.

Biochemistry of Lipids and Lipoprotein Metabolism

The major plasma lipids, cholesterol and triglyceride, are essential substrates for cell membrane formation and provide a source of energy in the form of free fatty acids.[8,10] The lipoprotein structure consists of cholesterol, triglyceride, phospholipid, and apolipoproteins and serves as a transport vehicle for lipids in plasma. Hyperlipidemia may be defined as an increased concentration of plasma lipids that are composed of cholesterol, triglycer-

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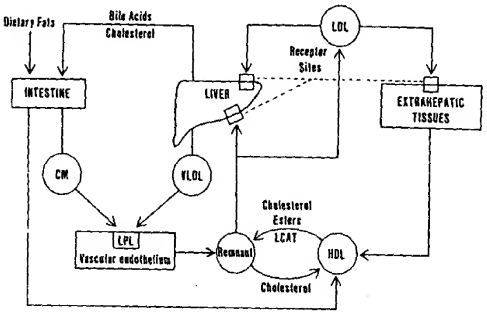
Figure 1. Summary of lipid and lipoprotein metabolism; where CM = chylomicron, VLDL = very low density lipoprotein, LDL = low-density lipoprotein, HDL = high-density lipoprotein, LPL = lipoprotein lipase, and LCAT = lecithin cholesterol acyltransferase.



ide, cholesterol ester, and phospholipid.[8] Hyperlipoproteinemia describes an increased concentration of the lipoprotein macromolecules that transport lipids in the plasma. More precise definitions of an increase in lipid concentrations are addressed later.

The lipid transport system involves a complex interaction between tissue receptors and lipoprotein molecules with the purpose of transferring lipids to and from tissue sites (Figure 1).[8,10,11] Briefly, dietary fats are solubilized in the intestine by bile salts and absorbed in intestinal mucosal cells where chylomicrons are formed. Chylomicrons are released into the plasma and partially metabolized by lipoprotein lipase in vascular endothelium to form a chylomicron remnant. The remnant is subsequently taken up by the liver and metabolized at specific receptor sites. The liver synthesizes very low density lipoprotein (VLDL) that is catabolized like the chylomicron by lipoprotein lipase to form an intermediate-density lipoprotein (IDL) or remnant particle. The IDL remnant may be catabolized in the liver or converted to low-density lipoprotein (LDL), the major transport protein for cholesterol. Low-density lipoproteins are catabolized in hepatic and extrahepatic tissues to release free cholesterol. High-density lipoprotein (HDL), synthesized in the liver and intestines, then acts as a receptacle for free cholesterol present in tissues. In addition HDL acts as a sort of dispatcher, transferring apolipoproteins between different lipoproteins. Catabolism of HDL ultimately occurs in the liver and kidney.

Low-density lipoproteins primarily consist of cholesterol, and their main function is to provide cholesterol to tissues. Consequently, LDL concentrations directly correlate with the risk of CAD. Conversely, although the composition of HDL is predominantly cholesterol, it acts as a receptacle for cholesterol from the tissues, and its plasma concentrations are inversely correlated with CAD risk.

For a more detailed discussion of lipoprotein metabolism, readers may refer to several excellent reviews.[8,11-13]

Although increased concentrations of triglyceride may cause pancreatitis or exacerbate diabetes mellitus, the association between increased concentrations of lipids and the risk of CAD is of primary concern.[4,14] Considerable controversy surrounds the role of triglyceride as an independent risk factor for CAD. Some evidence exists that a subgroup of hypertriglyceridemic patients with a specific elevation of apolipoprotein B may be at increased risk for CAD.[15] However, based on current knowledge, increased concentrations of triglyceride serve to identify patients with a coexistent elevation in cholesterol or underlying disease, such as diabetes mellitus, that may place them at risk for CAD.[14,15]

Increased serum cholesterol concentrations have been conclusively shown to be an independent risk factor for CAD.[1-3] Considerable controversy exists over when and how to treat patients with elevated cholesterol concentrations. The association between cholesterol concentration and risk is nonlinear, i.e., patients above the 95th percentile for serum cholesterol values are at a disproportionately greater risk than those below this level.[8] Treatment of patients above the 95th percentile reduces the incidence of CAD compared with untreated patients.[5,6] Further, preliminary evidence indicates that atherosclerosis can be halted or reversed in these same high-risk patients with aggressive treatment.[16-18]

However, several unanswered questions remain. The optimal level for serum cholesterol has not been precisely defined. The Pooling Project Research Group found a threshold between 200 and 220 mg/dL above which CAD risk sharply increased.[19] Results of the Multiple Risk Factor Intervention Trial (MRFIT) showed an increase in CAD risk at cholesterol concentrations above 175 mg/dL.[20] Although patients with serum cholesterol concentrations between the 50th and 95th percentile are likely to be at increased risk for CAD, the risks and benefits of treatment remain to be determined. In addition, the benefits of aggressive treatment on the progression or regression of atherosclerosis in low-risk patients are unclear. Unfortunately, because of prohibitive costs, these questions are not likely to be addressed in a large-scale controlled trial and probably will remain unanswered.

Etiology of Hyperlipidemia

Fredrickson and Lee[21] first suggested a classification of hyperlipoproteinemias based on the electrophoretic lipoprotein pattern. This scheme has been widely adopted and proves useful in classifying familial disorders. However, little information

Table 1.
Etiology of Hyperlipoproteinemia

Primary (genetic)
Receptor abnormality
Apolipoprotein abnormality
Enzyme abnormality
Apolipoprotein deficiency
Enzyme deficiency
Secondary
Diabetes mellitus
Pancreatitis
Hypothyroidism
Multiple myeloma
Alcohol ingestion
Obesity
Nephrotic syndrome
Pregnancy
Systemic lupus erythematosus
Drug induced
Dietary
High fat and cholesterol intake
Obesity

about the etiology of the lipid disorder is provided by this classification. The American Heart Association (AHA) in a consensus statement suggested a classification based on etiology that may be more useful when evaluating the patient.[8] It delineated three general categories that focus on the cause of disease: (1) primary or genetic, (2) secondary to other diseases or drugs, and (3) dietary. Specific examples for each category are listed in Table 1.

Patient Evaluation

Initial suspicion that a patient may be hyperlipidemic often occurs when an elevated cholesterol or triglyceride concentration is reported through a routine laboratory examination. Once hyperlipidemia is suspected, the patient should be thoroughly evaluated to confirm the diagnosis and determine appropriate therapy. Two major components of the patient evaluation are the history and physical examination and the laboratory examination. A complete history and physical examination should be performed with four goals in mind: (1) To evaluate whether factors that increase cardiovascular risk, e.g., smoking, hypertension, and obesity are present, (2) to determine if there is a family history of lipid disorders, (3) to exclude secondary causes of the disease, and (4) to examine whether xanthomas, abdominal pain, renal or liver disease, diabetes mellitus, or vascular disease is present.[8,9,11]

The cornerstone of the laboratory diagnosis of hyperlipidemia involves repeated measurements of serum or plasma cholesterol and triglyceride concentrations. Specimens should be obtained after an overnight fast of at least 10–12 hours to allow for adequate clearance of triglycerides from the plasma.[22] At least two determinations should be made on separate occasions to provide a reliable baseline. During this time, the patient should be on a constant diet to maintain a stable weight, and the patient should not be experiencing any acute illness.[22] For example, a myocardial infarction causes serum lipid concentrations to increase sharply and their return to baseline may not occur for weeks or months.

A careful medication history should be obtained because drug-induced hyperlipidemia is often not considered in the differential diagnosis. Estrogens were initially included in the Coronary Drug Project; however, with the discovery of increased cardiovascular morbidity, this phase of the study was discontinued.[23] More recently, oral contraceptive use has been associated with increased CAD risk.[24] Two studies specifically examined the relationship between oral contraceptive use and lipid abnormalities.[25,26] Both found that HDL cholesterol concentrations were directly related to estrogen dose while they were inversely related to progestin potency. Therefore, the choice of oral contraceptive should take into consideration potential lipid changes and the patient's CAD risk.

Various antihypertensive agents have been found to affect lipid and lipoprotein concentrations adversely. A recent review summarized these effects.[27] Thiazide diuretics significantly increased triglyceride concentrations by as much as 20% and total and LDL cholesterol while decreasing HDL cholesterol concentrations by 5% in both short- and long-term trials.[27,28] The effects on triglyceride and total cholesterol concentrations were sustained throughout the duration of the MRFIT program; however, the effects on LDL cholesterol concentration disappeared after the initial two years of the study.[28] Centrally acting α agonists appear to produce far less predictable changes, and to date no significant alterations in serum lipid concentrations have been identified.[27,28] β Blockers cause a significant increase in triglyceride and decrease in HDL cholesterol concentrations, and these effects appear to be potentiated when these agents are used in combination with diuretic drugs.[27,28] The magnitude of alteration may be more prominent with nonselective agents and may be completely absent with β blockers possessing partial-agonist activity.[27] The α-blocking activity of labetalol also may counteract the adverse effects of β blockade on lipid concentrations.[27] Prazosin, calcium antagonists, and angiotensin-converting enzyme inhibitors (i.e., captopril, enalapril) have not been found to have any important effects on serum lipid and lipoprotein concentrations.[27] These agents may be the drug of choice in hypertensive patients with pre-existing lipid abnormalities. Although the lipid changes induced by some antihypertensive agents may not produce overt hyperlipidemia, the beneficial effects of reduced blood pressure on CAD risk may be nullified.

The need for additional laboratory tests in the evaluation and diagnosis of hyperlipidemia is

somewhat controversial.[22,29] Previously lipoprotein electrophoresis or ultracentrifugation was recommended to quantify lipoprotein concentrations and identify the lipoprotein phenotype. However, each lipoprotein type represents several diseases so that phenotyping usually does not provide information about the etiology of the disorder.[29] In addition, electrophoresis and ultracentrifuge techniques require an expertise not available in all clinical laboratories.[22,29] These techniques are recommended in circumstances where a patient may have a familial disorder that cannot be diagnosed by history and physical examination.[29]

Although routine quantification of lipoproteins by electrophoresis is unnecessary, an approximation may be obtained by a simple calculation.[30] The presence of chylomicrons can be determined by examining a plasma sample after overnight storage at 4 °C. Chylomicrons appear as a creamy, white surface layer. In the absence of chylomicrons, VLDL carries the major portion of plasma triglyceride that exists in a relatively fixed proportion with cholesterol. Based on these assumptions, the cholesterol content may be estimated by dividing the plasma triglyceride concentration by 5. High-density lipoprotein cholesterol may be quantified by a relatively simple heparin–manganese precipitation technique.[31] Using this information, LDL cholesterol may be calculated by subtracting the sum of HDL cholesterol and triglyceride/5 from total cholesterol. To summarize, in the absence of chylomicrons, LDL and VLDL cholesterol may be calculated by simply measuring plasma triglyceride, cholesterol, and HDL cholesterol. This information along with the history and physical examination usually provides an accurate diagnosis in patients with hyperlipidemia.

The determination of HDL-cholesterol concentrations in the routine evaluation of hyperlipidemia has received increasing attention. Strong evidence supports an inverse correlation between HDL-cholesterol concentrations and risk of CAD.[32,33] Further, when fractionated into three components, the HDL-2 subfraction appears to correlate most closely with CAD risk.[11] An HDL-cholesterol concentration below the 5th percentile increases the risk of CAD by as much as eight times over that of a patient with an HDL-cholesterol concentration above the 95th percentile.[32,33] Despite this association, HDL-cholesterol determinations are of diagnostic value in only a few situations: (1) in patients with elevated plasma cholesterol concentrations that may be attributable to the HDL component alone and (2) to prompt aggressive intervention in the high-risk patients when HDL-cholesterol concentrations are low.[9,34] HDL-cholesterol concentrations may be increased by moderate alcohol intake, physical exercise, smoking cessation, weight loss, and oral contraceptives.[35] Interestingly, exercise and estrogen use appear to increase the HDL-2 fraction, while alcohol consumption affects the less important HDL-3 fraction.[36] The effects of antihypertensive drugs on HDL-cholesterol concentrations were previously discussed. Phenytoin and terbutaline have been reported to increase HDL-cholesterol concentrations while benzodiazepines may produce a modest decrease.[35,37]

A limitation to any biochemical determination is the accuracy and precision of the clinical laboratory. Large interlaboratory and intralaboratory differences in test results have been noted, especially with respect to cholesterol, HDL, and lipoprotein electrophoresis results.[22,38] Clinical laboratories may use different assay procedures resulting in substantial variations in tests results. For example, plasma cholesterol values are 3% lower than serum values.[39] Knowledge of the source of reference values for each biochemical test is important in evaluating the results. It must be stressed that the normal range of serum lipid concentrations merely represents a population mean plus or minus two standard deviations and does not define the risk of disease. Currently, a patient with a plasma cholesterol concentration above 200 mg/dL is at some increased risk for CAD.[9] The most current reference values for the U.S. population are taken from results of the Lipid Research Clinics Program.[40] This survey defined values that are higher for plasma cholesterol and lower for plasma triglyceride than previous reference values. A summary of reference values from the Lipid Research Clinics Program is presented in Table 2.

Management of the Patient with Hyperlipidemia

After the initial evaluation has been completed, patient management should be approached with specific goals in mind. The goals of therapy should be to (1) reduce cholesterol or triglyceride concentrations or both to below the 75th percentile, (2) modify co-existing risk factors, (3) individualize the treatment to patient, and (4) minimize any adverse effects. Specific interventions must be determined on the basis of patient age, gender, etiology of hyperlipidemia, presence of other risk factors, and degree of lipid abnormality.

In patients with secondary hyperlipidemia, treatment of the underlying disease represents the first step of therapy.[9,22] Hypothyroidism, diabetes mellitus, excessive alcohol intake, and other causes for hyperlipidemia should be managed. Often the lipid abnormality resolves itself with effective treatment of the underlying disorder. Interventions that address other CAD risk factors should be implemented at an early date, e.g., increase exercise, decrease or stop smoking, lose weight. Maintenance of ideal body weight is an important factor in control of the disease.[9] Lastly, the patient and family should be educated about the reasons for

Table 2.
Reference Values for Plasma Cholest rol and Triglyceride C ncentrations (mg/dL)[a]

Age (years)	Cholesterol			Triglyceride	
	50th Percentile	75th Percentile	95th Percentile	50th Percentile	95th Percentile
Men					
< 20	155	170	200	< 80	< 150
20–24	165	185	220	100	200
25–29	180	200	245	115	250
30–34	190	215	255	130	265
35–39	200	225	270	145	320
40–44	205	230	270	150	320
45–69	215	235	275	140	290
≥ 70	205	230	270	135	260
Women					
< 20	160	175	200	< 75	< 130
20–24	170	190	230	90	170
25–34	175	195	235	90	170
35–39	185	205	245	95	195
40–44	195	215	255	105	210
45–49	205	225	270	110	230
50–54	220	240	285	120	240
≥ 55	230	250	295	125	250

[a] Data adapted from reference 40 with permission.

these changes in lifestyle.

Dietary Therapy. The majority of patients with hyperlipidemia may be managed with dietary therapy alone, obviating the need for drugs.[7,8,22] This is especially true for patients with hypertriglyceridemia and secondary or dietary causes of hypercholesterolemia. The rationale behind dietary intervention is based on the effects of high cholesterol, fat, and caloric intake on lipid metabolism. It has been well demonstrated that excessive ingestion of these dietary components results in increased synthesis of LDL and VLDL and accelerated atherosclerosis.[41–43] Therefore, the goals of dietary therapy are to decrease the intake of cholesterol, fats (especially saturated fats) and calories, and maintain ideal body weight.[7,8]

Although many different dietary programs have been proposed to accomplish these goals, the most simplified approach has been developed by the AHA.[7] AHA has a three-phase diet that emphasizes a gradual reduction in cholesterol and fats with substitution of polyunsaturated for saturated fats. Phase I has been recommended for the general population and includes a decrease in cholesterol intake to under 300 mg daily, no more than 30% of the total calories as fats equally distributed between saturated, monounsaturated, and polyunsaturated, and an increase in complex carbohydrates. With recent changes in American dietary habits, many people have already achieved the goals of phase I. In phase II of the diet, cholesterol intake is reduced further to less than 250 mg per day and fats account for no more than 25% of the total calories. Phase III restricts fats to 20% of total calories, and cholesterol intake to 100–150 mg per day. Some patients experience an increase in VLDL triglyceride after a restriction in fats and an increase in carbohydrates. However, this is a transient response that usually disappears after a few months.[8,22]

Connor et al.[44] predicted a 14–21% decrease in plasma cholesterol concentrations when progressing from a standard diet through each phase of the AHA diet. However, reports from several multicenter studies have found more modest changes from dietary intervention. The Lipid Research Clinics trial used a diet that provided 400 mg/day of cholesterol and increased polyunsaturated fats and reported an overall 4.9% decrease in plasma cholesterol concentration with diet alone.[5,6] In the MRFIT study,[45] a diet aimed at reducing the intake of saturated fats to under 8% and cholesterol to below 250 mg produced an 8% decrease in total cholesterol. Recently, Arntzenius et al.[46] reported results from a two-year dietary intervention trial that used a diet similar to phase III of the AHA diet. Although the study lacked a control group for comparison, the results showed an average reduction of 10.1% in total cholesterol. Even though each of these studies used different dietary measures, the results indicate that there are limits to the magnitude of decrease in cholesterol levels that can be expected from dietary intervention. A summary of the results of some of these dietary intervention trials are reported in Table 3.[47–49]

Depending on the response to diet, age of patient, and risk factors, the patient may be advanced to successive phases of the diet until the target cholesterol level is reached. Each phase of the diet should be maintained for a minimum of four weeks to confirm patient compliance with the therapy. However, intervention for six months or more at the prescribed dietary phase may be necessary to obtain optimal results. A dietitian well trained in

Table 3.
Summary of Major Studies on Dietary Therapy

Reference Number	Number of Patients	Study Design	Therapy	Duration (years)	Outcome
1	1,900	Survey	Diet	20	Dietary cholesterol associated with serum cholesterol and CHD risk
45	12,866	Randomized	Diet, smoking, hypertension	7	No difference in mortality
47	5,000	Crossover	Diet	12	Decrease in CHD mortality in male patients
48	1,232	Randomized	Diet, smoking	5	47% decrease in infarction and sudden death
49	846	Double-blind randomized	Diet	8	No difference in mortality

counseling patients can provide invaluable assistance in instructing the patient and family about the diet and providing follow-up care.[14] Equally important is counseling the patient and family members about the risks and complications of CAD and the benefits that should be derived from diet modification and other lifestyle changes.[22]

Drug Therapy. Although most patients with hyperlipidemia can be adequately treated with diet alone, there are situations that necessitate the institution of drug therapy. Most patients with familial hypercholesterolemia require drug therapy to normalize plasma cholesterol concentrations.[8] Also, high-risk patients who do not adequately respond or are unwilling to comply with diet therapy may require the use of drugs. General guidelines for deciding to treat with drugs should take into account the age of the patient, the presence of other CAD risk factors, and the level of cholesterol or triglyceride after diet therapy has been instituted. As a general rule, patients who consistently have a plasma cholesterol concentration above the 95th percentile or a triglyceride concentration above 500 mg/dL after an adequate dietary trial should be considered for drug therapy.[14,22] Tables 4–6 provide a summary of drug effects, treatment recommendations, and cost comparisons.

Bile-Acid-Binding Resins. Cholestyramine and colestipol hydrochloride are nonabsorbable resins that bind bile acids in the intestinal lumen.[50,51] In so doing they effectively block the reabsorption of bile acids and reduce the bile-acid pool available to the liver. Shepherd et al.[52] showed that cholestyramine increases LDL catabolism by an effect on the LDL receptor. Hepatic bile-acid synthesis increases, requiring increased catabolism of cholesterol from LDL cholesterol. This results in a decrease in circulating and LDL cholesterol.

Bile-acid resins have been extensively evaluated for the treatment of hypercholesterolemia where reductions of 20% in total and LDL cholesterol have been noted.[5,6,16,52–54] A National Heart, Lung, and Blood Institute (NHLBI) study compared cholestyramine and diet versus diet alone in a five-year double-blind, randomized trial to determine the effects of treatment measured angiographically on progression of arteriosclerosis in patients with evidence of CAD[16] Patients received 24 g of cholestyramine daily in addition to dietary therapy, and almost 80% of patients demonstrated good compliance. A 17% decrease in total cholesterol and 26% decrease in LDL cholesterol were found in the treatment group versus decreases of 1% and 5% in the control group, respectively. In addition, a regression of atherosclerosis was shown by angiography in the treatment group.

The Lipid Research Clinics–Coronary Primary Prevention Trial (LRC-CPPT) evaluated type II hyperlipoproteinemic patients without evidence of CAD who had plasma cholesterol concentrations above the 95th percentile to test the efficacy of diet and cholestyramine.[5,6] Patients received diet therapy and either cholestyramine 24 g daily or placebo and were followed for up to seven years. Total cholesterol and LDL cholesterol decreased 8.5% and 12.6% more in the cholestyramine group than the placebo group. Triglycerides increased in the treatment group by 10.9% and in the control group by 8.8%. Coronary heart disease (deaths and nonfatal infarctions) decreased by 19%.

The results of this study were the basis for a National Institutes of Health recommendation calling for a population-wide modification in diet to reduce CAD.[9] However, the results have been criticized on several points.[20,55–58] The patients in the study were all men in a definite high-risk category. A 19% reduction in CAD was demonstrated but the absolute difference between the treatment and control groups was 1.6%. The reduction in total and LDL cholesterol was directly correlated with compliance with cholestyramine and was greatest in patients demonstrating complete compliance. Finally, patients in the treatment group experienced more noncardiac deaths caused by violence and suicide. For these reasons, the results should not be extrapolated as a basis for population-wide recommendations and should be viewed with some reservation.

Table 4.
Effects of Drug Therapy on Lipids and Lip proteins[a]

Drug	Mechanism of Action	Effects on Lipids	Effects on Lipoproteins	Comment
Cholestyramine and colestipol hydrochloride	↑ LDL catabolism	↓ Cholesterol	↓ LDL ↑ VLDL	Problem with compliance; binds many coadministered drugs; good in combination with bile-acid resins
Niacin	↓ LDL and VLDL synthesis	↓ Triglyceride and cholesterol	↓ VLDL, ↓ LDL, ↑ HDL	Problems with patient acceptance
Dextrothyroxine sodium	↑ LDL catabolism	↓ Cholesterol	↓ LDL	Demands caution in patients with heart disease
Clofibrate	↑ VLDL clearance	↓ Triglyceride and cholesterol	↓ VLDL and LDL, ↑ HDL	Possible long-term toxicity; only modest effects on cholesterol
Neomycin sulfate	↓ Cholesterol absorption	↓ Cholesterol	↓ LDL	Potentially ototoxic and nephrotoxic
Probucol	↑ LDL clearance	↓ Cholesterol	↓ LDL and HDL	Lowers HDL; modest efficacy
Gemfibrozil	↑ VLDL clearance ↓ VLDL synthesis	↓ Triglyceride and cholesterol	↓ VLDL ↑↓ LDL, ↑ HDL	Similar to clofibrate; long-term toxicity unknown
Mevinolin and compactin	↑ LDL catabolism	↓ Cholesterol	↓ LDL	Investigational; may be highly effective in familial disease

[a] The arrows indicate increases (↑) and decreases (↓) and LDL = low-density lipoprotein, VLDL = very low density lipoprotein; HDL = high-density lipoprotein.

Table 5.
Summary of Lipoprotein Phenotype and Suggested Drug Treatment[a]

Lipoprotein Phenotype	Lipoprotein Abnormality	Lipid Changes	Drug of Choice	Alternatives
I	Chylomicron	↑ Triglyceride	Not indicated	Not indicated
IIa	Low density	↑ Cholesterol	Bile-acid resins	Probucol, niacin, neomycin sulfate, compactin and mevinolin, in any combination with resins
IIb	Low and very low density	↑ Triglyceride, ↑ Cholesterol	Niacin	Gemfibrozil, clofibrate
III	Remnant	↑ Triglyceride, ↑ Cholesterol	Niacin	Gemfibrozil, clofibrate
IV	Very low density	↑ Triglyceride	Niacin	Gemfibrozil, clofibrate
V	Chylomicron and very low density	↑ Triglyceride, ↑ Cholesterol	Niacin	Gemfibrozil

[a] The arrows indicate increases (↑) and decreases (↓) in concentration.

Table 6.
Comparison of Drugs Used in the Treatment of Hyperlipidemia

Drug	Manufacturer	Dosage Forms	Usual Daily Dose	Maximum Daily Dose	Cost/Month ($)[a]
Cholestyramine (Questran)	Mead Johnson	4-g packets, bulk powder	8 g t.i.d.	32 g	136.44 61.67
Colestipol hydrochloride (Colestid)	Upjohn	5-g packets, bulk powder	10 g b.i.d.	30 g	59.44 57.06
Niacin	Various	50-, 100-, 250-, and 500-mg tablets; 125-, 250-, and 500-mg capsules	2 g t.i.d.	9 g	4.57–386.06
Probucol (Lorelco)	Merrell-Dow	250-mg tablets	500 mg b.i.d.	1 g	37.70
Dextrothyroxine sodium (Choloxin)	Flint	1-, 2-, 4-, and 6-mg tablets	6 mg every day	8 mg	17.20
Neomycin sulfate	Various	500-mg tablets	1 g b.i.d.	2 g	57.25
Clofibrate (Atromid-S)	Ayerst	500-mg capsules	1 g b.i.d.	2 mg	22.25
Gemfibrozil (Lopid)	Parke-Davis	300-mg capsules	600 mg b.i.d.	1.5 g	33.50

[a] Cost based on 1985 *Blue Book* average wholesale price for 30 days of usual daily dose.

Cholestyramine powder and colestipol beads must be mixed with a liquid before ingestion. Each formulation should be sprinkled on the surface of at least 120 mL of water or juice with care taken to avoid clumping. Since colestipol is odorless and tasteless, it may be preferable in some circumstances. A recipe for incorporating cholestyramine into cookies may overcome some taste and odor problems; however, the formulation has not been evaluated for stability and binding properties.[59] Four grams of cholestyramine and 5 g of colestipol hydrochloride are equivalent and may be given in divided doses before or with meals.[60] Some authors report that twice or even once daily dosing of these products is as well tolerated and efficacious as more frequent dosing.[61-63] The cholestyramine formulation contains tartrazine and should be used cautiously in aspirin-sensitive patients.

The majority of side effects reported with the resins involve the gastrointestinal tract.[5,6,16,52-54] Constipation, bloating, and heartburn most commonly occur, but abdominal pain, gas, nausea, and belching may also occur. In the LRC-CPPT these complaints tended to wane with prolonged therapy. Stool softeners or an increase in dietary fiber may alleviate the constipation.[64] The use of bile-acid resins may result in the malabsorption of fat-soluble vitamins, however, especially at doses above 24 g/day.[65] In the case of vitamin K, hypoprothrombinemia may result, although the administration of vitamin K will readily correct the deficiency.[66] Folic acid deficiency has been reported in children, sometimes requiring supplementation.[67] There are some reports of increased cholelithiasis with bile-acid resins; however, the incidence was no greater in the treatment group versus the control group in the LRC trial.[5,6]

There have been several reports of altered absorption of drugs concurrently administered with the resins. The drugs affected include warfarin, digitoxin, thyroxine, thiazides, and iron.[68-72] Digoxin bioavailability may also be affected by cholestyramine administration. Brown et al.[73] reported decreases in urinary excretion and serum concentrations of digoxin that were both temporally and dose related to cholestyramine therapy. Further studies by the same investigators showed that the effect was more prominent with the tablet formulation of digoxin than the capsule formulation.[74] Patients taking bile-acid resins concurrently with any other medication should be instructed to take the other drugs at least one hour before or four hours after a dose of resin.

Plasma triglyceride and VLDL concentrations often increase in patients after resin treatment is initiated.[5,16,43,65] The mechanism for this increase is not well understood but an increase in hepatic synthesis of VLDL occurs and may be responsible.[75] Occasionally, the addition of a second drug, such as niacin or clofibrate may be required if the triglyceride concentration remains elevated.

Niacin. Niacin (nicotinic acid) acts by inhibiting the synthesis and secretion of VLDL from the liver. By decreasing VLDL formation, subsequent production of LDL is also reduced.[76] Bile-acid excretion remains unchanged but the excretion of neutral sterols in the stool increases. Consequently, niacin is effective in decreasing both plasma cholesterol and triglyceride concentrations.[77,78] Niacin also increases HDL cholesterol by reducing its catabolism.[79,80] The complementary action of niacin and bile-acid resins to increase the excretion and decrease the absorption of sterols may account for the additive effects of this combination in hyperlipidemia.[64]

The principal use of niacin is for mixed hyperlipidemia or as a second-line agent in combination therapy for hypercholesterolemia.[64,81,82] Recent recommendations from the NIH consensus conference include niacin as the drug of choice or an alternate for treatment of hypertriglyceridemia.[14] As a single agent, niacin reduced cholesterol 9.9% and triglyceride 26.1% in the Coronary Drug Project.[83] Kane et al.[64] combined diet, colestipol, and niacin in patients with type II disease and found niacin alone produced a 20% decrease in cholesterol and almost a 40% decrease in triglyceride. Both LDL and VLDL concentrations were decreased while the HDL concentrations increased by 17%. When combined with colestipol, the combination produced a reduction of 45% or greater in plasma cholesterol concentration.[64,81] Hoeg et al.[82] treated type II patients with neomycin and niacin and found an 18% decrease in total cholesterol and 25% decrease in LDL cholesterol while HDL increased 32% when the effect of niacin was evaluated separately.[82] The combined therapy resulted in a decrease in total and LDL cholesterol by 36% and 45%, respectively.[82]

Niacin is available in tablet or sustained-release capsule formulations. Therapy should be initiated at a low dose (300–600 mg/day) and gradually increased by 300 mg per week to the maximum tolerated doses.[84] This slow titration is necessary to avoid the dose-related side effects of niacin.[84]

The most prominent side effect, intense facial flushing, occurs when therapy begins and with subsequent dosage increases and results from the release of prostaglandins causing capillary dilatation.[64,81] Tolerance to the flushing usually occurs after the patient has been maintained on a stable dose for several days; however, if the therapy is interrupted for a few doses, the flushing will promptly recur when the therapy is reinstituted.[85] Administering each dose with food or 300 mg of aspirin may alleviate the flushing.[64,81,85]

Dose-related increases in aspartate aminotransferase and alkaline phosphatase occur and have been related to increases in dose of more than 2.5 g per month.[64] Even though these changes usually resolve

with stabilization of the dose, liver enzyme test results should be routinely monitored. Hyperuricemia and glucose intolerance are common abnormalities but only present a problem in patients with diabetes mellitus or gout.[84,85] Skin rashes and pruritus frequently occur but they are usually not dose limiting.[85] Gastric irritation may be a problem, especially with higher doses; therefore, patients should be instructed to take the drug with food or antacid when gastrointestinal symptoms occur.[85]

Neomycin Sulfate. Neomycin sulfate has not been approved by the Food and Drug Administration (FDA) for treatment of hyperlipidemia. Despite this restriction, numerous studies have documented the lipid-lowering efficacy of neomycin.[86-89] Most authors suggest cautious use of neomycin because of concerns about toxicity; however, Samuel[90] suggested that neomycin sulfate be reconsidered for the treatment of hyperlipidemia because of the rare occurrence of side effects.

Neomycin reduces cholesterol absorption from the gastrointestinal tract, resulting in a reduction in total and LDL cholesterol.[87,90] The exact mechanism by which this occurs is unknown but may be related to altered cellular function, changes in colonic flora, or alterations in bile-acid metabolism.[87,90]

When compared with other drugs, neomycin produces equal or greater reductions in plasma cholesterol concentrations. Cholesterol decreased by 15–25% when neomycin was used alone for patients with type II hypercholesterolemia, and most of the decrease corresponded to an effect on LDL, since no effect was found on HDL cholesterol.[86,87] Samuel et al.[88] found a 29% decrease in cholesterol with neomycin sulfate versus 17% with clofibrate. When compared with niacin, neomycin alone reduced cholesterol and LDL cholesterol by 23% and 29%, respectively, and combined therapy reduced cholesterol 36% and LDL cholesterol 45%.[82] In this study, neomycin significantly reduced HDL cholesterol by about 7% when compared with diet alone.[82] These data provide support for the efficacy of neomycin sulfate alone or in combination for the treatment of hypercholesterolemia.

Gastrointestinal side effects most commonly complicate neomycin sulfate therapy. Up to half the patients experience diarrhea or abdominal cramps that usually resolve after a few weeks of continued therapy.[91] Monilial infections, staphylococcal colitis, and resistant coliform bacteria have been reported rarely.[90] Neomycin may interfere with digoxin absorption, necessitating administration of the drug at least one hour before the neomycin.[85,91,92]

Neomycin effectively reduces total and LDL cholesterol with the advantage of a convenient twice daily dosage schedule. Despite concerns about toxicity and lack of FDA approval, some authors recommend neomycin sulfate as the initial therapy for hypercholesterolemia.[87,90] Long-term experience with a large number of patients will be necessary before this recommendation can be widely supported.

Probucol. Probucol reduces plasma cholesterol and LDL cholesterol without affecting VLDL.[93-95] The precise mechanism for this effect is unknown, but it is likely that probucol increases the clearance of LDL from the blood without affecting its metabolism.[95] HDL concentrations also decrease, probably the result of reduced synthesis of apolipoproteins.[93]

Most studies using probucol have been in patients with type II hypercholesterolemia. The reduction in total cholesterol has exceeded 10% in all cases and has been as high as 21% after three years of treatment in a report from Taylor et al.[93,96-99] Mellies et al.[93] also reported an 8.4% reduction in LDL and a 26% decrease in HDL with probucol. The reduction in HDL cholesterol was consistent with a 32% reduction in HDL reported by Dujovne et al.[100] Probucol causes a consistent decrease in total cholesterol that is accompanied by decreases in LDL and HDL cholesterol.

Side effects with probucol are infrequent and generally minor. Taylor et al.[99] observed side effects in 21 of 588 patients, which consisted mostly of nausea, diarrhea, and abdominal discomfort. Diarrhea was also reported by Dujovne et al.[100] and may be attributable to increased excretion of bile acids. Other adverse effects reported include visual disturbances, headache, dizziness, chest pain, neuritis, weight loss, and constipation.[98,99]

Probucol is effective in moderately reducing total and LDL cholesterol and apparently has a low incidence of minor side effects. However, the importance of its negative effect on HDL cholesterol and the impact on CAD risk remains unclear. For this reason, probucol should not be considered a first-line agent for treatment of hypercholesterolemia at this time.

Dextrothyroxine Sodium. Cholesterol undergoes hepatic conversion to bile acid under the influence of an α-hydroxylation reaction in the liver.[85] In addition, LDL receptors in the liver and other tissues serve an important role by removing cholesterol from LDL. Thyroxine has been found to stimulate the enzymatic reaction and to increase the number of hepatic LDL receptors.[85] Recognizing these effects, dextrothyroxine was developed with the hope that it would have minimal metabolic and cardiac effects associated with other thyroid preparations. These effects persist with dextrothyroxine and severely limit its usefulness. Interestingly, Bantle et al.[101] compared thyrotropin-suppressing doses of dextrothyroxine and levothyroxine in euthyroid patients and found no difference in cholesterol-lowering effects or heart rate. These results confirmed previous findings from a report of hypothyroid patients.[102]

Dextrothyroxine sodium reduces total and LDL cholesterol by as much as 20%.[103] Investigators from the Coronary Drug Project (CDP) reported a decrease in serum cholesterol and triglyceride of 8% and 12%, respectively, when compared with placebo.[104] Unfortunately, excessive cardiovascular mortality in the CDP necessitated dropping dextrothyroxine from the study.[104]

Side effects include precipitation or enhancement of cardiac arrhythmias and angina pectoris, elevation of liver enzymes, increased metabolic rate, and suppression of thyroid function.[85,104] Concurrent propranolol therapy has been suggested to control the cardiac effects of dextrothyroxine; however, the availability of other less toxic agents precludes the use of this regimen under most circumstances.[103] Dextrothyroxine sodium is contraindicated in patients with a history of CAD or arrhythmias. Patients on oral anticoagulants may experience potentiation of anticoagulation secondary to displacement from binding sites by dextrothyroxine.[105] Also, increased sensitivity to the effects of digitalis glycosides has been reported.[106] The 2- and 6-mg tablets of dextrothyroxine sodium contain tartrazine and should be avoided in patients with a history of allergies.

In summary, dextrothyroxine sodium effectively decreases total and LDL cholesterol; however, the severity of its side effects limits its usefulness to younger patients without evidence of heart disease. When its use is deemed appropriate, the starting dose is 2 mg daily and the dosage should be increased by 1-mg increments at monthly intervals to tolerance.[85]

Clofibrate. Clofibrate was the first drug approved by the FDA for the treatment of hyperlipidemia. Although it was initially a popular choice in the treatment of lipid disorders, large-scale clinical trials uncovered a high incidence of noncardiovascular morbidity and mortality.[83,107-109] With the development of new drugs and recommendations for managing lipid disorders, clofibrate can no longer be considered the drug of choice for any of these diseases. Despite these limitations, clofibrate still has a role as an alternative agent alone or in combination with other drugs.

Clofibrate increases lipoprotein-lipase activity in extrahepatic tissues, enhancing the catabolism of VLDL.[110,111] Cholesterol synthesis in the liver is decreased and LDL catabolism increases.[111] These effects contribute to a significant decrease in triglyceride and VLDL, more modest decreases in total and LDL cholesterol, and a modest increase in HDL cholesterol. Clofibrate most effectively decreases triglyceride concentration in type III and IV hyperlipoproteinemias, while combinations with other drugs are effective in type II disorders.[11]

In the CDP, clofibrate produced a 22% decrease in triglyceride; however, cholesterol was only reduced by 6.3%.[83] A second large trial using clofibrate, 1.6 g/day reported a 9% decrease in serum cholesterol.[108,109] When Kane et al.[64] compared clofibrate, niacin, and colestipol, clofibrate had no significant effect on total, HDL, and LDL cholesterol when added to colestipol therapy. However, triglyceride was decreased 28% from baseline with the combination. A small trial of clofibrate in hypertriglyceridemia found 32% and 38% reductions in triglyceride and VLDL, respectively.[112] Unfortunately, this was accompanied by a 26% increase in LDL cholesterol, although total and HDL cholesterol concentrations were unchanged.

Despite its potential benefits, there are several reservations about the long-term use of clofibrate. In both the CDP and World Health Organization (WHO) trials, noncardiac deaths were increased, and a follow-up report of the WHO trial found an increase in mortality from all causes including CAD in the clofibrate group. The CDP trial also reported an increase in pulmonary embolism, angina, and arrhythmias plus a twofold increase in gallbladder disease when comparing clofibrate with the placebo group.[83,107] Other reported adverse effects include a loss of libido, breast tenderness, elevations in liver enzyme tests, myositis, and thromboembolism.[83,108] Clofibrate has been found to potentiate the action of warfarin, possibly increasing the effects by as much as 50%.[113]

To summarize, clofibrate produces its most substantial effects on triglyceride and VLDL concentrations. Modest decreases in total cholesterol occur but long-term changes in HDL are usually not apparent.[114] With the questions remaining about its long-term safety, clofibrate should probably be reserved for patients who cannot tolerate other agents and have failed to respond positively to dietary intervention.

Gemfibrozil. A recent addition to the lipid-regulating group of drugs, gemfibrozil has properties similar to clofibrate. In common with clofibrate, gemfibrozil stimulates lipoprotein lipase to increase VLDL lipolysis and decrease plasma triglyceride.[112,115] Unlike clofibrate, gemfibrozil also may reduce the synthesis and secretion of VLDL.[112,115] It causes a significant increase in HDL concentrations; however, it also increases LDL in some patients while decreasing LDL in others.[112,115]

In clinical trials, gemfibrozil has been shown to be effective in lowering triglyceride and VLDL concentrations in patients with type II and type IV hyperlipoproteinemias.[112,115-119] Both triglyceride and VLDL concentrations are consistently reduced by 40–60% regardless of the type of lipid disorder.[112,116,118] In contrast, the effects on total cholesterol, LDL, and HDL concentrations vary with the specific disorder. Manninen et al.[116] evaluated type II patients and found a 16% and 23% decrease in cholesterol and LDL concentrations, respectively. Others have generally noted more modest reductions of less than 10%.[118] However, in patients with

a disorder primarily in triglyceride metabolism (type IV), total cholesterol is usually unchanged and LDL increases 10–12%.[11,15] Regardless of the underlying disorder, HDL-cholesterol concentrations routinely increase by 17–31%.[112,116,118] Based on these results gemfibrozil is most appropriate for lipid disorders involving increases in triglyceride and VLDL concentrations.

The adverse effects observed with gemfibrozil are similar to those seen with clofibrate. The most often reported effects are rash in 2% of patients and abdominal discomfort.[116,118] An elevated eosinophil count has been reported in 1% of patients and is usually associated with rash.[118] Transient elevations in transaminases and alkaline phosphatase may occur in up to 4.5% of patients.[118] Glucose intolerance has been observed and may increase insulin requirements in diabetic patients.[118] Because of problems with cholelithiasis during clofibrate therapy, gemfibrozil has been closely observed for similar problems. A trend toward an increase in gallstones was reported by Samuel;[118] however, more widespread use of the drug is required to confirm this observation. Gemfibrozil reportedly potentiates the anticoagulant effects of warfarin, requiring close observation when given concurrently.[120]

In summary, gemfibrozil may be considered the drug of choice for severe hypertriglyceridemia because of its beneficial effects on VLDL, HDL, and triglyceride concentrations. It also may be useful in combination with bile-acid resins in the treatment of mixed hyperlipidemia. Compared with clofibrate and niacin, gemfibrozil has the advantage of a lower incidence of adverse effects. The increase in LDL cholesterol may limit its use in patients with co-existing increases in cholesterol.[112,118]

Compactin and Mevinolin. Two promising new compounds for the treatment of hypercholesterolemia, compactin and mevinolin, are currently undergoing clinical investigation.[121–126] Both act by inhibiting 3-hydroxyl-3-methylglutaryl-coenzyme A reductase (HMG CoA reductase), the enzyme that catalyzes the rate-limiting step in cholesterol synthesis.[121,122]

To date, most studies with these compounds have been in patients with a type II disorder.[121–124] When the effects of compactin or mevinolin are considered alone, total cholesterol concentrations reportedly decrease 22–33% while LDL-cholesterol concentrations drop 27–34%.[121–125] Triglyceride concentrations decrease with compactin (36%); however, Illingworth found no effect on triglycerides with mevinolin.[122–124] When the effects of HMG CoA reductase inhibitors are considered alone, HDL cholesterol concentrations are not affected, although Mabuchi et al.[123] found that the decrease in HDL produced by cholestyramine was maintained when compactin was added to the therapy.

Patient complaints with these drugs are minimal but biochemical abnormalities have been observed.[122,124–126] Transient increases in creatine phosphokinase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase concentrations and decreases in uric acid concentration have all been reported with short- and long-term therapy. All abnormalities return to normal after the drug is discontinued. Headache has been reported in two patients, and gastrointestinal side effects have not been reported.[124]

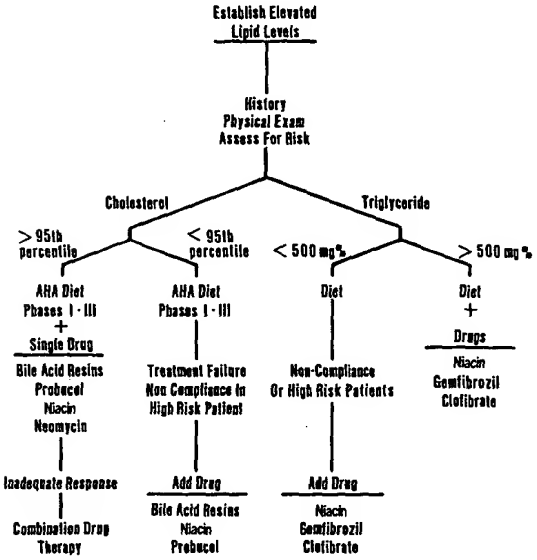
The HMG CoA reductase inhibitors represent promising additions to the therapeutic armamentarium of hyperlipidemia. They are likely to be most useful in type II disorders in combination with other drugs. Extensive clinical trials are needed to establish the long-term efficacy and safety of these compounds.

Patients with type II familial hypercholesterolemia often have an inadequate response despite appropriate diet and single drug therapy. In these patients, combinations of drugs have frequently been found to be effective. Niacin, when combined with colestipol or neomycin, produces additive effects on total and LDL cholesterol.[64,81,82] Similarly, neomycin, probucol, compactin, and mevinolin demonstrate additive effects when combined with bile-acid resins.[89,100,123,124] In these instances combination therapy has reduced total cholesterol concentrations by 50–100% more than single drug therapy. Unfortunately, the reduction in HDL cholesterol caused by probucol is maintained with the combination of probucol and colestipol.[100] Despite the dramatic responses reported with combination therapy, it should be reserved for patients with severe hypercholesterolemia because of the potential for increased side effects.

Other Therapies. The failure of diet and drug therapy to lower cholesterol concentrations adequately in patients with severe familial hypercholesterolemia has fostered research on alternative forms of therapy. Partial ileal bypass has been used effectively in heterozygous patients resistant to or noncompliant with drug therapy.[127] Ileal bypass surgery removes the site of bile-acid reabsorption, depleting the bile-acid pool and increasing the catabolism of cholesterol. Indirect evidence suggests that the number of hepatic and extrahepatic LDL receptors increases as a secondary response. This procedure reduces LDL cholesterol concentrations by as much as 40%; however, some patients may become refractory over time, and it is ineffective in homozygous type II patients.[127–129]

Portacaval shunts have been used in refractory homozygous type II patients, where they cause a decrease in the formation of LDL.[130,131] Concentrations of LDL cholesterol can be expected to decrease by 10–20%, and larger reductions have occasionally been reported.[130,131]

Figure 2. Treatment algorithm for hyperlipidemia.



Plasma exchange has recently gained some attention for its efficacy in lowering LDL cholesterol and reversing the signs of CAD.[132,133] Thompson et al.[132] reported a resolution of angina in two patients treated by plasma exchange and demonstrated a shift of cholesterol from tissue stores to the plasma. The same group reported on 22 patients with severe hypercholesterolemia including 20 patients with heterozygous and homozygous disease who were treated for up to five years with plasma exchange.[133] Overall, effective control of hypercholesterolemia was found with the exchange procedure when it was used at 1- to 2-week intervals. Plasma exchange appears promising in the heterozygous or homozygous patient who is symptomatic with progressive CAD when other treatments have been ineffective.

Most recently, liver transplantation has been investigated as a method for treating homozygous hypercholesterolemia.[134] The rationale for the use of this procedure is based on the knowledge that homozygous patients are almost totally devoid of hepatic LDL receptors. Therefore, pharmacological interventions aimed at increasing the number of LDL receptors are ineffective. A single report of liver transplantation in a homozygous patient found a dramatic resolution of hypercholesterolemia.[134] Widespread use is limited by the inadequate number of organ donors; however, liver transplantation may be the most promising procedure for managing the rare patient with homozygous type II hypercholesterolemia.

Recommendations for Managing Hyperlipidemia

Hyperlipidemia should be managed systematically using information about the association between elevated lipid concentrations and CAD, patient risk factors, and limitations of both diet and drug therapy. Management should be approached with specific goals in mind. Current NIH and AHA recommendations are controversial and a consensus in the medical community has not been reached on a population-wide adoption of these guidelines. However, it is clear that patients above the 50th percentile for plasma cholesterol concentrations are at some increased risk for CAD and should benefit from intervention.

Accordingly, patients with plasma cholesterol concentrations above the 50th percentile should be encouraged to adopt a diet designed to reduce saturated fat and cholesterol intake. Patients with plasma cholesterol concentrations above the 75th percentile should be prescribed a diet designed to lower their levels below this percentile. Patients at high risk for CAD should be considered for drug therapy if diet therapy is not effective. Patients with cholesterol concentrations above the 95th percentile should receive intensive dietary intervention aimed at lowering the plasma cholesterol concentrations below the 75th percentile. If dietary therapy is ineffective, these patients should be treated with drug therapy to achieve the desired reduction. With these guidelines in mind, a thorough evaluation of the patient including history, physical and laboratory examination, and assessment of risk for CAD is necessary. Specific treatment must be approached with the individual in mind. Patient age, family history, risk of CAD, and risks versus benefits of therapy must be considered. Using this information, an appropriate therapeutic plan may be developed. Figure 2 provides an algorithm outlining one approach to patient management.

Conclusion

Strong evidence supports the role of increased cholesterol concentrations as an independent risk factor for CAD. Evidence that elevated triglyceride concentrations are also an independent risk factor remains questionable. Clearly, patients above the 95th percentile for cholesterol are at high risk for CAD and should receive some form of treatment. However, the majority of patients with hyperlipidemia have cholesterol concentrations between the 50th and 95th percentile. Treatment should be approached with a firm understanding of the relationship between CAD and hyperlipidemia, and specific goals of therapy should be developed that are adaptable to the individual patient. The limitations of diet and drug therapy should be recognized before initiating therapy, and the patient and family should be made aware of the rationale for treatment.

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102b support for fenofibrate

Fenofibrate causes—
↓ apoE transcription
↓ apoE mRNA levels
(tissue-specific)

≠ Gemfibrozil does not

Fibrates Influence the Expression of Genes Involved in Lipoprotein Metabolism in a Tissue-Selective Manner in the Rat

Bart Staels, Arie van Tol, Toni Andreu, and Johan Auwerx

The influence of different fibrates on apolipoprotein metabolism was investigated. Administration of fenofibrate provoked a dose-dependent decrease in plasma cholesterol concentration that was already evident after 1 day. Intestinal apolipoprotein (apo) A-I and apo A-IV mRNA levels remained fairly constant. In contrast, liver apo A-I, apo A-II, and apo A-IV mRNA levels decreased in a dose-dependent fashion, which was associated with a lower transcription rate of the apo A-I but not the apo A-II gene. The decline in hepatic apo A-I, apo A-II, and apo A-IV mRNA had already started after 1 day and was associated with a drop in plasma apo A-I and apo A-IV concentrations. Plasma apo E had already decreased after 1 day of fenofibrate, whereas apo B initially remained constant and increased only after 14 days of fenofibrate at the highest dose. Hepatic and intestinal apo B mRNA contents and liver, heart, kidney, and testis apo E mRNA contents were only marginally affected after treatment with fenofibrate. Liver low density lipoprotein receptor mRNA levels rose slightly after a 3-day administration of the highest dose of fenofibrate. Both clofibrate and gemfibrozil had effects comparable to those of fenofibrate on liver and intestinal apolipoprotein mRNA levels except for liver apo A-II mRNA, which decreased only marginally. Compared with fenofibrate, clofibrate caused similar changes in plasma cholesterol, apo A-I, apo A-IV, and apo E concentrations, whereas gemfibrozil increased plasma cholesterol and apo E without changing apo A-I and apo A-IV concentrations. In conclusion, all fibrates influence the expression of the apo A-I, apo A-II, and apo A-IV genes but not the apo B and apo E genes in a tissue-selective manner. After clofibrate and fenofibrate but not after gemfibrozil, these alterations are reflected by similar changes in plasma apolipoprotein concentrations. (*Arteriosclerosis and Thrombosis* 1992;12:286–294)

KEY WORDS • atherosclerosis • gene expression • hyperlipidemia • hypolipidemic drugs • steroid hormone receptors

Fibrates are a class of hypolipidemic drugs widely used in the treatment of diet-resistant hyperlipidemia. In humans, fibrates decrease plasma triglyceride and/or cholesterol concentrations.[1–5] In laboratory animals such as the rat, fibrates are extremely effective in lowering plasma cholesterol and occasionally plasma triglyceride concentrations.[6–11] Although very little is known about the exact mechanism of action of these drugs, the decreases in plasma cholesterol and triglyceride levels are classically thought to be the result of a decrease in hepatic cholesterol synthesis[7,12–14] and the induction of lipoprotein lipase in peripheral tissues,[15–20] respectively. In addition, fibrates have been shown to be potent inducers of the β-oxidation system of fatty acids in the liver and, to a lesser extent, in the intestine and kidney in rodents.[21–24] These compounds act by inducing the expression of several genes that code for peroxisomal enzymes in the liver.[25–30] The induction of these enzymes results in a strong proliferation of peroxisomes and hepatomegaly and may ultimately lead to the development of hepatocarcinomas in animals treated for a prolonged period.[31,32]

Interestingly, a receptor activated by peroxisomal proliferators (such as fibrates) and belonging to the erb-A/steroid receptor gene superfamily has been recently cloned.[33] Receptors belonging to this family activate the transcription of ligand-inducible genes after binding as ligand–receptor complexes to specific DNA elements in the promotor region of genes. It is, therefore, tempting to speculate that fibrates might use a similar ligand-activated receptor to transmit their effect on gene expression.

In view of the strong effects of fibrates on lipoprotein metabolism, the aim of this study was to determine whether fibrates influence the expression of genes that code for proteins involved in lipoprotein metabolism, such as the different apolipoproteins or the low density lipoprotein (LDL) receptor, and whether the observed effects on plasma cholesterol and apolipoprotein concentrations could be explained by the alterations in gene expression in different tissues such as the liver or intestine. The results from these studies demonstrate

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that all fibrates tested affect the expression of the apo A-I, apo A-IV, and, to a lesser extent, the apo A-II genes in the liver but not the intestine. However, these effects on gene expression correlate with the pronounced decrease of the corresponding plasma apolipoprotein concentrations only after clofibrate and fenofibrate but not after gemfibrozil administration.

Methods

Animals and Treatments

Male Wistar rats (90 days old) were treated for different periods of time with fenofibrate (Laboratoires Fournier, Daix, France), clofibrate (Sigma Chemical Co., St. Louis, Mo.), or gemfibrozil (Warner-Lambert Co., Ann Arbor, Mich.) mixed at the indicated concentrations (wt/wt) in standard rat chow. The food intake of the rats was recorded every 2 days throughout the treatment period. None of the treatments caused major changes in the amount of food consumed by the animals. Because each rat consumed approximately 20 g of chow per day, doses of 0.5%, 0.3%, 0.05%, and 0.005% fibrate ([wt/wt] mixed in rat chow) corresponded to 320, 190, 32, and 3 mg/kg body wt/day. At the end of the experiments, the animals were fasted overnight and killed at approximately 9 AM by exsanguination while under ether anesthesia. Blood was collected in EDTA-containing tubes, and plasma was either used within 1 day for flotation of plasma lipoproteins or stored at −20°C for determination of cholesterol and apolipoprotein levels. Liver, heart, testis, and kidneys were removed immediately, rinsed with 0.9% saline, and frozen in liquid nitrogen. The intestine was removed and rinsed with ice-cold 0.9% saline, and the epithelium was scraped off and frozen in liquid nitrogen.

Measurement of Plasma Cholesterol and Apolipoprotein Concentrations

Plasma total cholesterol was measured as described previously.[34] Plasma apo A-I, apo A-IV, and apo E concentrations were measured by electroimmunoassay, exactly as described previously.[35] Plasma apo B was measured by radial immunodiffusion as described.[36]

Total plasma lipoproteins were isolated by flotation through a potassium bromide gradient.[37] The lipoproteins obtained by flotation were lyophilized and then delipidated for 10 minutes at 95°C in 4% sodium dodecyl sulfate (SDS), and the apolipoproteins were separated by electrophoresis by SDS–polyacrylamide gel electrophoresis (PAGE) (15% resolving gel, 4% stacking gel).[38] Equal volumes of each sample were loaded to estimate relative changes in apolipoproteins. The different apolipoproteins were identified by comparing their mobility with purified apolipoproteins and by their apparent molecular weights.[38]

RNA Analysis

RNA was prepared by the guanidine isothiocyanate/cesium chloride procedure from liver, kidney, heart, testis, and intestinal epithelium of individual animals.[39] Northern and dot-blot hybridizations of total cellular RNA were performed as described previously.[34] Care was taken to apply equal amounts of total RNA to all dot and Northern blot filters. The following apolipoprotein probes were used: a rat apo A-I cDNA probe[34]; a rat apo A-II cDNA clone[40]; a 42-base oligonucleotide probe constructed complementary to bases 73–114 of rat apo A-IV[37]; a rat apo E cDNA clone[34]; a rat 1.2-kb apo B cDNA probe[41]; and a 0.3-kb *Bam*HI restriction fragment of the human LDL receptor clone, pLDLR-3.[42] A chicken β-actin cDNA clone[43] was used as a control. cDNA probes were labeled by nick translation (apo A-I, apo A-II, apo B, and apo E) or random-primed labeling (β-actin and LDL receptor; Boehringer Mannheim, Mannheim, FRG). Oligonucleotide probes (apo A-IV) were 5′-labeled using T4 polynucleotide kinase. Filters were hybridized to 1.5×10^6 cpm/ml of each probe as described.[34] They were washed in 500 ml of 0.5× saline–sodium citrate buffer (SSC) and 0.1% SDS for 10 minutes at room temperature and twice for 30 minutes at 65°C and subsequently exposed to x-ray film (X-OMAT-AR, Kodak Co. Inc., Rochester, N.Y.). Autoradiograms were analyzed by quantitative scanning densitometry (LKB 2202 Ultrascan laser densitometer, LKB, Bromma, Sweden) as described.[34] None of the treatments caused significant changes in liver, intestinal, heart, testis, or kidney β-actin mRNA levels, therefore indicating that the observed effects of fibrates on apolipoprotein gene expression are specific and not due to changes in the liver weights of the rats.

Isolation of Nuclei and Transcriptional Rate Assay

Nuclei were prepared from livers of untreated rats and from livers of rats treated for 14 days with fenofibrate (0.5% [wt/wt] in rat chow) exactly as described by Gorski et al.[44] Transcription run-on assays were performed as described by Nevins.[45] Equivalent amounts of labeled nuclear RNA were hybridized for 36 hours at 42°C to 5 μg of purified cDNAs immobilized on Hybond-C Extra filters (Amersham, Arlington Heights, Ill.). The following cDNA probes were spotted: a rat apo A-I cDNA probe[34]; a rat apo A-II cDNA clone[40]; and a rat apo E cDNA clone.[34] As a control, 5 μg of vector DNA was also applied to the filter. After hybridization, filters were washed at room temperature for 10 minutes in 0.5× SSC and 0.1% SDS and twice for 30 minutes at 65°C and subsequently exposed to x-ray film (X-OMAT-AR, Kodak). Autoradiograms were analyzed by quantitative scanning densitometry (LKB 2202 Ultrascan laser densitometer).

Statistical Methods

Analysis of variance was used to evaluate the results of the dose–response and time-course experiments. If significance was attained, contrast statements were used to compare different dose and time groups. After checking whether variances were equal, the appropriate two-tailed unpaired Student's *t* test was used to evaluate differences between means in experiments with clofibrate and gemfibrozil.

Results

Time-Dependent Effects of Fenofibrate

Administration of fenofibrate mixed at 0.5% (wt/wt) in rat chow caused a small decline in body weight after 7 and 14 days, whereas liver weights had already increased after 1 day and rose further after 3 and 7 days (Table 1). Plasma cholesterol concentrations dropped

TABLE 1. Time-Dependent Influence of Fenofibrate on Body and Liver Weights and Plasma Cholesterol Concentrations in Rats

Time (days)	Body weight (g)	Liver weight (g)	Cholesterol (mg/100 ml)
0	318±8*	9.4±0.4†	80±16‡
1	315±11*‡	12.3±0.8‡	34±7†
3	310±11*‡	15.4±0.4*	32±6†
7	305±8‡	19.2±1.0§	50±15†
14	284±5†	18.9±1.0§	49±17†

Values represent mean±SD.
Adult male rats (n=3) received fenofibrate (0.5% [wt/wt] mixed in rat chow) for the indicated periods of time. Body and liver weights were recorded at the end of the treatment period. Plasma cholesterol concentrations were measured as described in "Methods."
Statistically significant differences (by analysis of variance; $p<0.05$) are observed between values followed by different symbols.

TABLE 2. Dose-Dependent Influence of Fenofibrate on Body and Liver Weights and Plasma Cholesterol Concentrations in Rats

Dose (%)	Body weight (g)	Liver weight (g)	Cholesterol (mg/100 ml)
0	318±8*	9.4±0.4†	80±16*
0.005	317±15*	9.4±0.2†	70±13*†
0.05	316±17*	13.4±0.9*	45±8†
0.5	284±5†	18.9±1.0‡	49±17†

Values represent mean±SD.
Adult male rats (n=3) were treated with the indicated dose (percent [wt/wt] in rat chow) of fenofibrate for 14 days. Body and liver weights were recorded at the end of the treatment period. Plasma cholesterol concentrations were measured as described in "Methods."
Statistically significant differences (by analysis of variance; $p<0.05$) are observed between values followed by different symbols.

maximally after 1 day of fenofibrate and remained fairly constant thereafter (Table 1).

Hepatic apo A-I mRNA levels fell to less than 50% of control after 1 day of fenofibrate and decreased to less than 10% of control after 7 days (Figure 1A). In the liver, apo A-II mRNA levels had already dropped to less than one fourth (Figure 1B), and apo A-IV mRNA levels fell to less than 20% of controls after 1 day of fenofibrate (Figure 1C). Intestinal apo A-I (Figure 1A) and apo A-IV (Figure 1C) mRNA levels, on the other hand, remained fairly constant throughout the treatment period. In plasma the concentrations of apo A-I and apo A-IV showed similar patterns: both apolipoproteins showed a maximal decrease after 3 days and remained low thereafter (Figure 1D).

After 1 day of treatment with fenofibrate, liver apo B and apo E mRNA levels decreased slightly by approximately 25% (Figures 2A and 2B). After 7 and 14 days, however, only apo B mRNA remained lower (Figure 2A), whereas apo E mRNA levels returned to levels comparable to those before treatment (Figure 2B). Intestinal apo B mRNA levels were not significantly influenced during treatment (Figure 2A). In plasma, apo E concentrations decreased after fenofibrate, whereas apo B remained fairly constant during the first week and showed an increase after 14 days (Figure 2D). Hepatic LDL receptor mRNA rose nearly 50% after 3 days of fenofibrate and remained slightly elevated thereafter (Figure 2C).

Dose-Dependent Effects of Fenofibrate

The body weights of the animals decreased only at the highest dose of fenofibrate tested, whereas liver weights had already increased after treatment with an intermediate dose and showed a further rise at the highest dose (Table 2). Plasma cholesterol concentrations tended to decrease at a dose of 0.005%, and a maximal effect was already attained at 0.05% fenofibrate (Table 2).

Hepatic apo A-I mRNA levels decreased in a dose-dependent fashion, whereas intestinal apo A-I mRNA levels remained constant (Figure 3A). Treatment with fenofibrate maximally decreased liver apo A-II and apo A-IV mRNA levels at a dose of 0.05% (Figures 3B and 3C). The changes in liver apo A-I and apo A-II mRNA levels, as observed after dot-blot hybridization, were confirmed after Northern blot hybridization (Figure 4). Intestinal apo A-IV mRNA was not significantly influenced at any of the doses tested (Figure 3C). Plasma

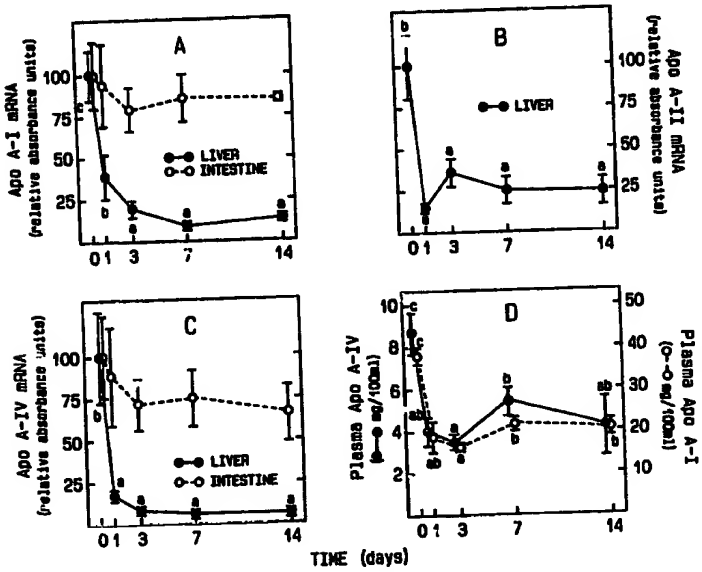

FIGURE 1. *Line plots showing the influence of duration of treatment with fenofibrate on liver and intestinal apolipoprotein (apo) A-I* (panel A), *apo A-II* (panel B), *and apo A-IV* (panel C) *mRNA levels and on plasma apo A-I and apo A-IV concentrations* (panel D). *Adult male rats were treated for the indicated number of days with fenofibrate (0.5% [wt/wt] mixed in rat chow). Plasma apolipoprotein concentrations and liver and intestinal mRNA levels were measured and expressed as described in "Methods." Statistically significant differences* ($p<0.05$ *by analysis of variance) are observed between values followed by different letters. Each value represents mean±SD of three animals.*

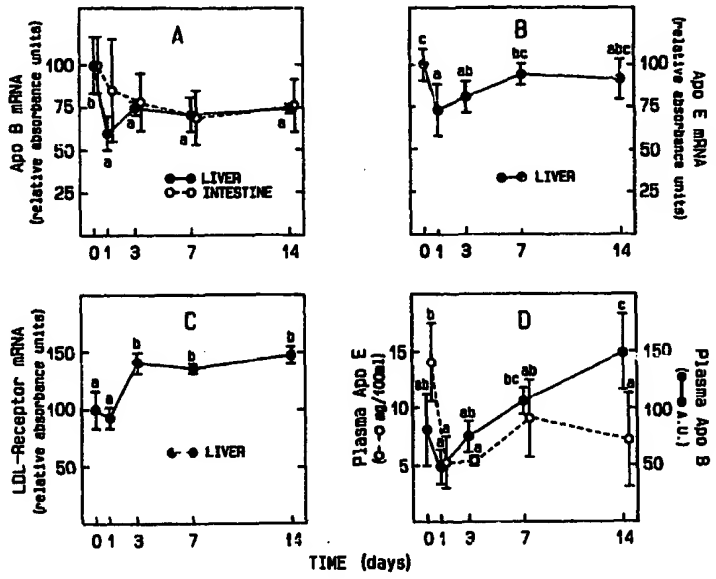

FIGURE 2. *Line plots showing the influence of duration of treatment with fenofibrate on liver and intestinal apolipoprotein (apo) B* (panel A), *apo E* (panel B), *and low density lipoprotein (LDL) receptor* (panel C) *mRNA levels and on plasma apo B and apo E concentrations* (panel D). *Animals and treatments were exactly as described in the legend to Figure 1. Plasma apolipoprotein concentrations and liver mRNA levels were measured and expressed as described in "Methods." Results were expressed as described in the legend to Figure 1. A.U., absorbance units.*

apo A-I and apo A-IV concentrations reflected the changes in hepatic mRNA levels and decreased in a dose-dependent manner (Figure 3D).

Liver apo B mRNA levels decreased slightly after a dose of 0.05% fenofibrate, whereas intestinal apo B mRNA levels were not significantly influenced (Figure 5A). Fenofibrate did not influence liver apo E mRNA levels (Figures 4 and 5B), whereas LDL receptor mRNA levels increased only after the highest dose tested (Figure 5C). In plasma, apo E concentrations had already dropped at an intermediate dose of fenofibrate, whereas apo B increased only at the highest dose tested (Figure 5D).

Influence of Fenofibrate on Hepatic Apolipoprotein A-I, Apolipoprotein A-II, and Apolipoprotein E Gene Transcription Rates

To determine whether the decrease in liver apo A-I and apo A-II mRNA levels was a consequence of a decrease in mRNA synthesis rates, nuclear run-on experiments were performed on nuclei prepared from livers of untreated control rats or rats treated with fenofibrate (0.5% [wt/wt]) for 14 days. The transcription rate of the apo A-I gene decreased approximately 10-fold in livers from rats treated with fenofibrate compared with control livers (Figure 6). In contrast, hepatic apo A-II gene transcription did not change at all after fenofibrate treatment, whereas hepatic apo E gene transcription decreased slightly after fenofibrate (Figure 6).

Influence of Fenofibrate on Extrahepatic Apolipoprotein E Gene Expression

Because treatment with fenofibrate lowered plasma apo E concentrations drastically without having major effects on liver apo E gene expression, it was investigated whether changes in extrahepatic apo E gene

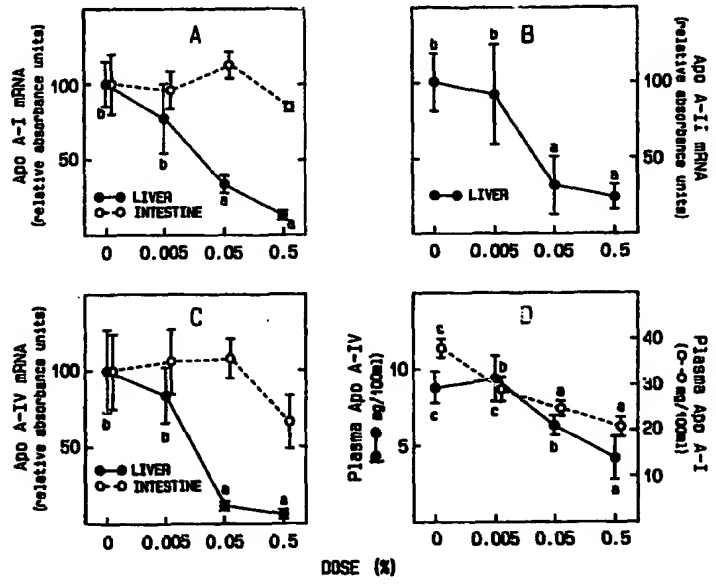

FIGURE 3. *Line plots showing the influence of treatment with different doses of fenofibrate on liver and intestinal apolipoprotein (apo) A-I* (panel A), *apo A-II* (panel B), *and apo A-IV* (panel C) *mRNA levels and on plasma apo A-I and apo A-IV concentrations* (panel D). *Adult male rats were treated for 14 days with the indicated doses of fenofibrate (wt/wt, mixed in rat chow). Plasma apolipoprotein concentrations and liver and intestinal mRNA levels were measured and expressed as described in "Methods." Statistically significant differences ($p<0.05$ by analysis of variance) are observed between values followed by different letters. Each value represents mean±SD of three animals.*

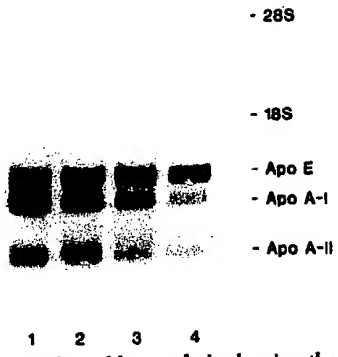

FIGURE 4. *Northern blot analysis showing the influence of different doses of fenofibrate on mRNA levels for apolipoprotein (apo) A-I, apo A-II, and apo E. RNA was prepared from livers of rats treated with different doses (lane 1, control; lane 2, 0.005%; lane 3, 0.05%; lane 4, 0.5% [wt/wt] in rat chow) for 14 days. Agarose gel electrophoresis of representative RNA samples and hybridizations were performed as described.[46] The localization of the 18S and 28S rRNAs are indicated on the autoradiograph.*

expression contributed to the observed effects on plasma apo E concentrations. Administration of fenofibrate produced a small but statistically significant decrease in testis and kidney apo E mRNA content, whereas heart apo E mRNA levels remained unchanged (Table 3). A small decrease in kidney apo E mRNA levels was observed after 7 days of fenofibrate (Table 4), whereas testis apo E mRNA levels decreased only after 14 days. However, the overall time-dependent effect was not statistically significant because of the large variation of the response after 1 day of treatment.

Influence of Treatment With Clofibrate and Gemfibrozil on Lipid and Apolipoprotein Parameters

Finally, it was investigated whether the effects observed after treatment with fenofibrate were general effects of fibrates or whether they were limited to fenofibrate only. Therefore, male rats were treated with either one of two other fibrates, clofibrate or gemfibrozil. Administration of clofibrate or gemfibrozil did not influence the body weights of the animals, whereas liver weights increased to an extent similar to that observed after treatment with fenofibrate at a dose of 0.05%–0.5% (Tables 2, 5, and 6).

Treatment with clofibrate reduced plasma cholesterol levels to 50% of the concentrations in the control rats, and plasma apo A-I, apo A-IV, and apo E dropped to two thirds of controls (Table 5). These changes in plasma apolipoprotein levels, as measured by immunoassay, were confirmed after SDS-PAGE of lipoproteins obtained by flotation from plasma of control and clofibrate-treated rats (Figure 7). Plasma apo B concentrations did not change significantly after clofibrate (Table 5). Liver apo A-I, apo A-II, and apo A-IV mRNA levels decreased after clofibrate, but the decrease in apo A-II mRNA was much less pronounced than that after fenofibrate (Table 5). Liver apo B, apo E, and LDL receptor mRNA levels and intestinal apo A-I, apo A-IV, and apo B mRNA levels were not significantly influenced by clofibrate (Table 5).

In contrast, administration of gemfibrozil increased plasma cholesterol and apo E concentrations, whereas plasma apo A-I, apo A-IV, and apo B concentrations remained unchanged (Table 6). However, liver apo A-I and apo A-IV mRNA levels decreased to 30% and to less than 10% of controls, respectively, whereas liver apo A-II and apo B mRNA levels dropped only marginally (Table 6). Intestinal apo A-I and apo B mRNA levels remained unchanged, whereas intestinal apo A-IV and liver LDL receptor mRNA levels tended to increase, but the effect was not statistically significant because of the large variation in response between animals (Table 6). Liver apo E mRNA levels did not change after gemfibrozil (Table 6).

Discussion

At present, relatively little is known about the mechanism through which fibrates alter plasma lipid and

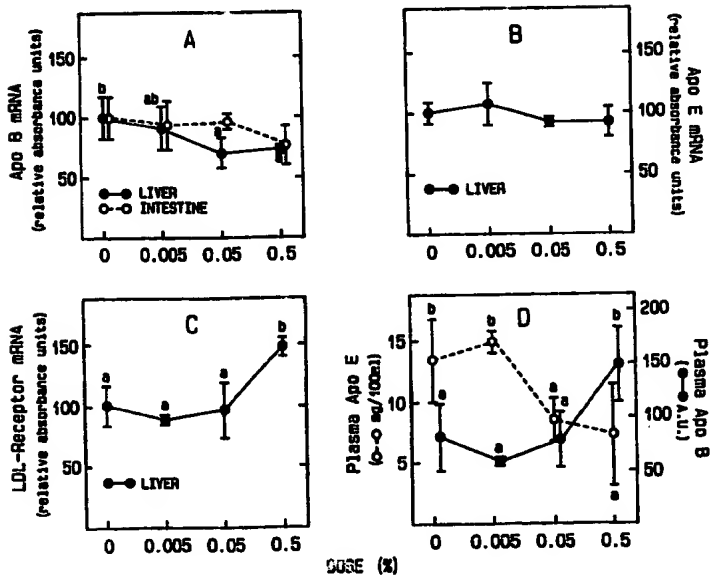

FIGURE 5. *Line plots showing the influence of treatment with different doses of fenofibrate on liver and intestinal apolipoprotein (apo) B* (panel A), *apo E* (panel B), *and low density lipoprotein (LDL) receptor* (panel C) *mRNA levels and on plasma apo B and apo E concentrations* (panel D). *Animals and treatments were exactly the same as described in the legend to Figure 1. Plasma apolipoprotein concentrations and liver mRNA levels were measured and expressed as described in "Methods." Results were expressed as described in the legend to Figure 3. A.U., absorbance units.*

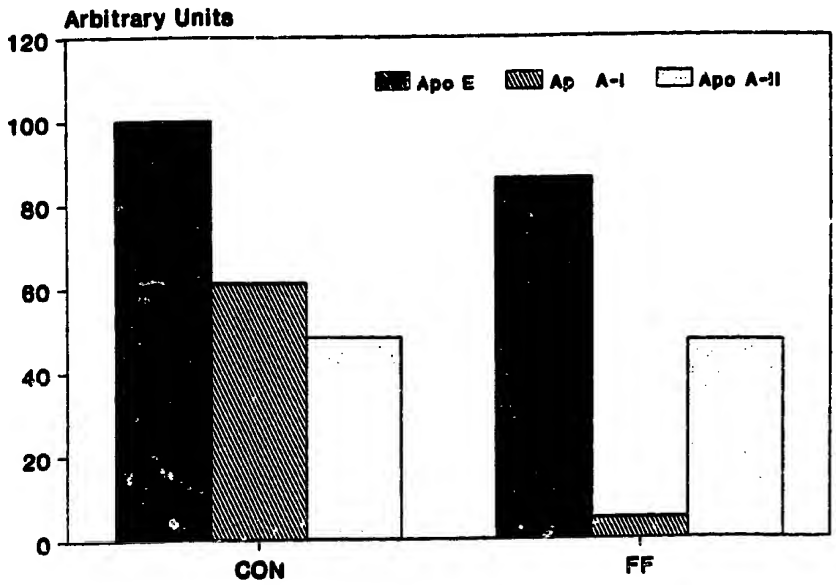

FIGURE 6. *Nuclear run-on transcription rate assay for hepatic apolipoprotein (apo) A-I, apo A-II, and apo E. Relative transcription rates were determined in nuclei from livers of untreated control rats (CON) and rats treated with fenofibrate (FF; 0.5% [wt/wt]) for 14 days. Nuclei were isolated, and nuclear run-on assays were performed as described in "Methods." Values as determined by laser densitometric scanning of autoradiograms of filters are expressed relative to the transcription rate of the apo E gene in control nuclei and represent the mean of two independent run-on assays.*

apolipoprotein concentrations. The results from these studies demonstrate for the first time that fibrates may act by selectively influencing the expression of genes involved in lipoprotein metabolism in the rat liver.

On the one hand, apo A-I, apo A-IV, and, to a lesser extent, apo A-II mRNA levels decrease after administration of fibrates. This decrease in mRNA steady-state levels is associated with a lowered transcription rate for the apo A-I gene but not for the apo A-II gene. Therefore, the expression of the apo A-II gene appears to be regulated at a posttranscriptional level, which may contribute to the variation in response of the apo A-II gene observed after treatment with different fibrates. In addition, the differential effects of clofibrate and fenofibrate, as opposed to gemfibrozil, on plasma apolipoprotein concentration but not on apolipoprotein gene expression suggest that fibrates may differentially affect apolipoprotein production at a posttranscriptional or translational level.

On the other hand, apo E and apo B mRNA levels are only marginally affected by fibrates. However, plasma apo E decreases after clofibrate and fenofibrate but increases after gemfibrozil. This increase in plasma apo E after gemfibrozil is in agreement with previous observations of cholesterol-fed rats treated with this drug.[47] Several mechanisms can be invoked to explain the lowered plasma apo E levels after clofibrate and fenofibrate. First, the decrease in plasma apo E may reflect an increased clearance of apo E–containing lipoproteins from plasma by the LDL receptor, whose mRNA levels are slightly increased in fenofibrate-treated liver. However, because the induction of LDL receptor gene expression trails the fall in plasma cholesterol and apo E levels by 2 days, it seems more likely that the pronounced decrease in plasma cholesterol causes an upregulation of LDL receptor gene expression instead of the opposite. Second, apo E production may be lowered. The decreased apo E gene expression in kidney and testis may contribute to the fall in plasma apo E concentrations. However, it is unlikely that the rather small decrease in apo E mRNA levels in these tissues is entirely responsible for this decrease, as the liver produces the majority of plasma apo E[48] and apo E gene expression is not affected in this organ. Finally, it is likely that apo E synthesis is regulated at posttranscriptional or translational levels by fibrates. Indeed, hepatic lipoprotein secretion has been shown to be

TABLE 3. **Dose-Dependent Influence of Fenofibrate on Extrahepatic Apolipoprotein E mRNA Levels in Rats**

Dose (%)	Heart (RAU)	Testis (RAU)	Kidney (RAU)
0	100±35	100±6*	100±5†
0.005	80±25	91±14*†	75±9‡
0.05	119±20	72±7‡	63±15‡
0.5	116±21	80±4‡	65±5‡

Values represent mean±SD.
RAU, relative absorbance units.
Adult male rats (n=3) were treated with the indicated dose (percent [wt/wt] in rat chow) of fenofibrate for 14 days. RNA was prepared, and apolipoprotein E mRNA levels were measured as described in "Methods."
Statistically significant differences (by analysis of variance; $p<0.05$) are observed between values followed by different symbols.

TABLE 4. **Time-Dependent Influence of Fenofibrate on Extrahepatic Apolipoprotein E mRNA Levels in Rats**

Time (days)	Heart (RAU)	Testis (RAU)	Kidney (RAU)
0	100±35	100±6	100±5
1	144±73	101±13	95±44
3	108±20	94±6	79±17
7	113±57	91±7	66±2
14	116±21	80±4	65±5

Values represent mean±SD.
RAU, relative absorbance units.
Adult male rats (n=3) received fenofibrate (0.5% [wt/wt] mixed in rat chow) for the indicated periods of time. RNA was prepared, and apolipoprotein E mRNA levels were measured as described in "Methods."
None of the treatments were statistically significant (by analysis of variance; $p<0.05$).

decreased in rats treated with fenofibrate and ciprofibrate[7] and clofibrate has been shown to lower hepatic very low density lipoprotein production and triglyceride secretion,[9,46] whereas gemfibrozil increases hepatic apo E synthesis and secretion.[49]

The increased plasma apo B concentrations, along with the unchanged liver and intestinal apo B mRNA levels, are remarkable, as liver LDL receptor mRNA levels are elevated in fenofibrate-treated rats. These observations suggest that fenofibrate may influence apo B production at a cotranscriptional or a posttranscriptional level. At present, studies are being undertaken to determine whether this drug regulates liver and/or intestinal apo B mRNA editing.

In contrast to their effects on liver apolipoprotein gene expression, intestinal mRNA levels remain unchanged or change in the opposite direction after treatment with fibrates. These observations extend our previous studies that show that apolipoprotein gene expression is much more susceptible to regulation in the liver than in the intestine.[34,37,41,50] Indeed, both liver apo A-I and apo A-IV gene expressions are regulated in a tissue-selective manner after treatment with several hormones, including corticosteroids,[50] thyroid hormones,[37,41] and estrogens,[34] with the liver being much more responsive than the intestine. This suggests that the expression of apolipoprotein genes is constitutive in the intestine, whereas expression in the liver is more regulated.

Finally, because of the peroxisomal proliferation observed after fibrate administration in rodents,[21-24] liver weights increase dramatically. These observations raise the question as to whether the effects of fibrates on apolipoprotein gene expression are mediated via their effects on peroxisomal proliferation and, hence, liver weight. Several lines of evidence, however, argue against this possibility. First, the brisk onset (1 day) of the changes in apolipoprotein gene expression suggest that fibrates may act directly on apolipoprotein gene expression. Second, the decreased transcription rate of the apo A-I gene in isolated nuclei points to a direct effect of fenofibrate on the expression of this gene. Finally, β-actin mRNA levels and transcription rates remain unchanged after administration of fibrates.

In conclusion, these studies show that fibrates have major effects on the expression of selective apolipoprotein genes in the rat liver but not in the intestine. Furthermore, the distinct effects of fenofibrate, clofi-

Table 5. Influence of Clofibrate on Body and Liver Weights, Plasma Cholesterol and Apolipoprotein Concentrations, and Liver and Intestinal mRNA Levels

Parameter	Clofibrate	
	Control	Treated
Body weight (g)	342±33	345±28
Liver weight (g)	9.9±1.0	13.8±1.4*
Cholesterol (mg/100 ml)	62±10	33±2*
Apo A-I		
Plasma (mg/100 ml)	33±5	21±1*
Liver mRNA (RAU)	100±18	45±12*
Intestinal mRNA (RAU)	100±14	103±5
Apo A-II		
Liver mRNA (RAU)	100±5	72±18*
Apo A-IV		
Plasma (mg/100 ml)	12±1	8±1*
Liver mRNA (RAU)	100±25	16±3*
Intestinal mRNA (RAU)	100±10	88±6
Apo B		
Plasma (AU)	96±25	61±21
Liver mRNA (RAU)	100±30	66±24
Intestinal mRNA (RAU)	100±11	86±11
Apo E		
Plasma (mg/100 ml)	9±1	6±1*
Liver mRNA (RAU)	100±6	107±5
LDL receptor		
Liver mRNA (RAU)	100±16	73±28

Values represent mean±SD.

Apo, apolipoprotein; LDL, low density lipoprotein; RAU, relative absorbance units; AU, absorbance units.

Adult male rats (n=4) were treated with clofibrate (0.3% [wt/wt] in rat chow) for 14 days. Body and liver weights were recorded at the end of the treatment period. Plasma cholesterol and apolipoprotein concentrations and liver and intestinal mRNA levels were measured as described in "Methods."

Statistically significant differences (by t test) from controls are indicated by an asterisk ($p<0.05$).

Table 6. Influence of Gemfibrozil on Body and Liver Weights, Plasma Cholesterol and Apolipoprotein Concentrations, and Liver and Intestinal mRNA Levels

Parameter	Gemfibrozil	
	Control	Treated
Body weight (g)	320±6	318±19
Liver weight (g)	9.4±0.3	16.2±1.8*
Cholesterol (mg/100 ml)	67±18	110±5*
Apo A-I		
Plasma (mg/100 ml)	42±4	41±8
Liver mRNA (RAU)	100±32	30±10*
Intestinal mRNA (RAU)	100±14	99±5
Apo A-II		
Liver mRNA (RAU)	100±15	78±31
Apo A-IV		
Plasma (mg/100 ml)	9±1	8±2
Liver mRNA (RAU)	100±21	8±1*
Intestinal mRNA (RAU)	100±20	172±43
Apo B		
Plasma (AU)	78±7	94±15
Liver mRNA (RAU)	100±2	92±4*
Intestinal mRNA (RAU)	100±13	139±26
Apo E		
Plasma (mg/100 ml)	13±2	23±2*
Liver mRNA (RAU)	100±6	96±14
LDL receptor		
Liver mRNA (RAU)	100±18	172±62

Values represent mean±SD.

Apo, apolipoprotein; LDL, low density lipoprotein; RAU, relative absorbance units; AU, absorbance units.

Adult male rats (n=4) were treated with gemfibrozil (0.5% [wt/wt] in rat chow) for 14 days. Body and liver weights were recorded at the end of the treatment period. Plasma cholesterol and apolipoprotein concentrations and liver and intestinal mRNA levels were measured as described in "Methods."

Statistically significant differences (by t test) from controls are indicated by an asterisk ($p<0.05$).

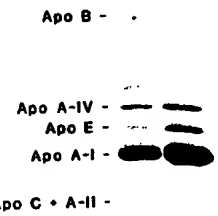

FIGURE 7. *Influence of clofibrate on plasma apolipoprotein levels. Adult male Wistar rats were treated with clofibrate (0.3% [wt/wt] in rat chow for 14 days; lane 1) or without (lane 2). Plasma lipoproteins (d<1.21 g/ml) were isolated and delipidated, and apolipoproteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis as described in "Methods."*

brate, and gemfibrozil on plasma apolipoprotein concentrations but not on liver apolipoprotein mRNA levels suggest that in addition to the effects of these drugs on gene expression, additional mechanisms appear to be operative. These observations warrant more detailed exploration of the effects of fibrates on gene expression in different animal species as well as in humans.

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Enabl. issues
- ApoE protects against atherosc.
- Deficient mice are more susc. to art. even on low-fat diet.

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Proceedings of the Australian Physiological and Pharmacological Society 1996 Symposium: Physiology of Lipid Transport

USE OF GENE-MANIPULATED MODELS TO STUDY THE PHYSIOLOGY OF LIPID TRANSPORT

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SUMMARY

1. *In vivo* and *in vitro* gene-manipulated models were used to study the metabolism of chylomicron remnants. Transgenic mice expressing human apolipoprotein (Apo) A1 or E4, gene knock-out mice deficient in ApoE or low density lipoprotein (LDL) receptors and antisense gene inhibition in HepG2 cells were used to evaluate the effect of gene manipulations on the metabolism of chylomicron remnants.

2. Mice transgenic for human ApoE4 showed accelerated clearance of chylomicron-like emulsions when animals were fed a low-fat diet. When challenged by a high-fat diet, remnant clearance in ApoE4 transgenic mice was delayed, as in normal or non-transgenic controls. However, unlike normal non-transgenic controls, in ApoE4 transgenic mice high density lipoprotein (HDL)-cholesterol levels remained high after high-fat feeding, which probably protected the animals from the development of atherosclerosis.[1] In contrast, clearance of chylomicron-like lipid emulsions was not affected by the over-expression of human ApoAI in transgenic mice.

3. Gene knock-out mice deficient in ApoE or deficient in the LDL receptor were used to show that ApoE and LDL receptors are both essential for the normal, fast catabolism of chylomicron remnants by the liver.[2] In the absence of the LDL receptor, an alternative ApoE-dependent pathway operates to clear chylomicrons from the plasma, with significantly delayed catabolism.

4. Antisense gene inhibition techniques were used to suppress the expression of syndecan, a core protein of heparan sulfate proteoglycan, in HepG2 cells. Remnant uptake in cells transfected with the antisense oligodeoxynucleotide complementary to a 20 nucleotide sequence upstream of the initiation site of syndecan cDNA markedly reduced the uptake of chylomicron remnant.

Key words: antisense, apolipoprotein, cholesteryl oleate, chylomicron remnant, chylomicron-like lipid emulsions, fractional clearance rate, heparan sulfate proteoglycan, lipolysis, oligodeoxynucleotide, transgenic mice, triolein.

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INTRODUCTION

The use of genetically manipulated models has greatly assisted investigations into the understanding of the physiology of lipid transport. Transgenic and homologous recombination techniques have allowed researchers to alter the genotype of an animal in a precise manner and to study the resultant change in phenotype. Transgenic mice over-expressing the genes for human apolipoproteins (Apo) and receptors and gene knock-out mice deficient in these proteins have increased our understanding of lipid transport. This in turn has led to an improved understanding of disease mechanisms in relation to atherosclerotic cardiovascular disease.

Over the past few years, many important conclusions have been drawn from studies of transgenic animals expressing human apolipoproteins. For example, the high expression of ApoAI and elevated high density lipoprotein (HDL) concentrations in ApoAI transgenic mice protect the animals against the development of atherosclerosis.[3] In contrast, transgenic mice expressing high levels of Apo(a)[4] and mice expressing high levels of human lipoprotein (a) (Lp(a)) particles,[5,6] are more susceptible to diet-induced atherosclerosis.

Although atherosclerosis is related to postprandial hyperlipidaemia, current understanding of lipid transport from the site of absorption to the site of utilization is insufficient to assess the quantitative role of chylomicron remnants in atherogenesis in comparison with other lipoproteins. In an attempt to elucidate factors involved in the hepatic uptake of chylomicron remnants from plasma, we have used transgenic mice expressing ApoE4, gene-knock-out mice deficient in ApoE or low density lipoprotein (LDL) receptor and hepatoma cells mutated by antisense gene-targeting as models for *in vivo* and *in vitro* studies.

METHODS

For the *in vivo* study, lymph chylomicrons were prepared from donor Wistar rats as previously reported.[7] Lipid emulsions mimicking lymph chylomicron were prepared[8] to contain [^{14}C]-triolein and [^{3}H]-cholesteryl oleate (CO), [^{14}C]-CO alone or fluorescent cholesteryl esters as tracers and the emulsions were injected intravenously into mice as reported previously.[2] The lipid emulsion did not contain any exogenous proteins, but immediately after injection into plasma the apolipoproteins of the recipient animals associated with the emulsion. Blood samples were taken from the orbital sinus at intervals for the measurement of radioactivity in plasma by scintillation counting.[1] The clearance rate of the emulsion was calculated from the decline of radioactivity in the plasma. Clearance of emulsion CO measures particle removal as remnants, while clearance of emulsion triolein measures lipolysis plus particle removal. In several previous studies, the clearances of lipid emulsions and lymph chylomicrons have been found to be comparable.[8,9,10]

Alternatively, animals were kept in a closed compartment and the expired $^{14}CO_2$ was collected for scintillation counting to measure the catabolism of remnants by the liver.[11] The intracellular pathway of remnants in the liver of the animal was followed by viewing confocal images of liver sections taken at time intervals after the injection of fluorescently labelled emulsions.[2]

To measure *in vitro* remnant uptake in tissue culture, remnants were harvested from the plasma of functionally hepatectomized rats injected with fluorescent emulsions.[12] HepG2 cells were transfected with synthetic antisense oligodeoxynucleotide (ODN) complimentary to syndecan, a core protein of liver heparan sulfate proteoglycan (HSPG). Control cells were transfected with synthetic sense ODN. After transfection, cells were incubated for 5 min at 37°C with an aliquot of 50 μL fluorescent remnants, washed five times with Hank's balanced salt solution (37°C) and then incubated with an equal amount of non-labelled remnants for 1 min. Cells were then fixed with 4% paraformaldehyde in 0.1 mol/L cacodylate buffer for 10 min at 4°C. Cover slips were removed from the culture dish and were mounted on slides with aquamont for examination with a confocal scanning microscope (BioRad MRC-1000; Hemel Hempstead, UK) with lens Plan Apo 60X (NA 1.40). Binding and uptake of remnants were assessed by computer analysis of the confocal images using the NIH image/68K 1.6 programme (distributed by the NIH of USA).

RESULTS AND DISCUSSION

Chylomicron clearance and atherosclerosis in transgenic mice expressing human apolipoproteins

Transgenic mice were generated from embryos injected with a cosmid containing the entire human ApoE4 gene driven by the human ApoA1 promoter to elevate the levels of transcript in the liver.[1] Emulsions labelled with [^{14}C]-triolein to trace the effect on lipolysis and [^{3}H]-CO to follow the clearance of emulsion remnants were injected. The clearance rates of both labels were compared with those in non-transgenic control animals. Our results show that mice transgenic for ApoE4 enhanced clearance ($P < 0.001$) of both [^{14}C]-triolein and [^{3}H]-CO from the plasma in animals consuming a low-fat diet (4% fat lab chow). If these animals were challenged with a high-fat, high-cholesterol diet (15% fat, 1% cholesterol and 0.5% sodium cholate), lipolysis of emulsion triglyceride was accelerated due to increased lipoprotein lipase activity (low-fat diet 6.3 U/mL to high-fat diet 9.2 U/mL plasma with male mice in each group), but remnant clearance was not improved in transgenic mice.[1] The decreased LDL receptor expression was probably sufficient to account for the slower remnant clearance in these mice. As shown in Fig. 1, compared with mice consuming a low-fat, control diet (lanes 1–4), the expression of LDL receptor decreased by 44% in mice consuming the high-fat diet for 3 weeks (lanes 5–7). Low density lipoprotein receptors were not detected in mice after they had consumed the high-fat diet for 18 weeks (lanes 9–12).

In transgenic mice HDL-cholesterol levels remained high after consuming the high-fat diet for 3 weeks (71 to 74 mg/dL; $n = 56$), unlike in control mice where HDL-cholesterol dropped significantly after consumption of the diet (68 to 52 mg/dL; $n = 36$). To test whether the high HDL-cholesterol level in transgenic mice was protective against the development of atherosclerosis, we assessed the atherosclerotic lesions in the aorta of the non-transgenic compared with transgenic mice consuming the high-fat diet for 14 weeks. When lesions in the aorta were quantified, transgenic mice showed a decrease in lesions, suggesting that they were protected from atherosclerosis.[1] The mechanism for this protective effect is still unclear, but could possibly be attributed to the ApoE-dependent, LDL receptor-independent pathway of remnant uptake by the animal livers.[2]

Clearance of emulsion triglyceride and emulsion CO increased somewhat in transgenic mice expressing high levels of ApoAI, but the difference was not significant (results not shown), suggesting that ApoAI has little effect on the clearance of chylomicrons. Confirmation of this finding requires studies of chylomicron clearance in ApoAI knock-out mice.

Chylomicron metabolism in gene knock-out mice deficient in ApoE

Colonies of ApoE knock-out mice and LDL receptor knock-out mice were established from progenitor stocks obtained from the Jackson Laboratories (Bar Harbor, MA, USA). Mice were derived and bred by sibling matings to obtain animals homozygous for null mutation. Animals were tested for the null mutation by western blotting for plasma ApoE and by PCR genotyping of the LDL receptor gene.[13]

As shown in Fig. 2a, the rate of emulsion triglyceride removal in

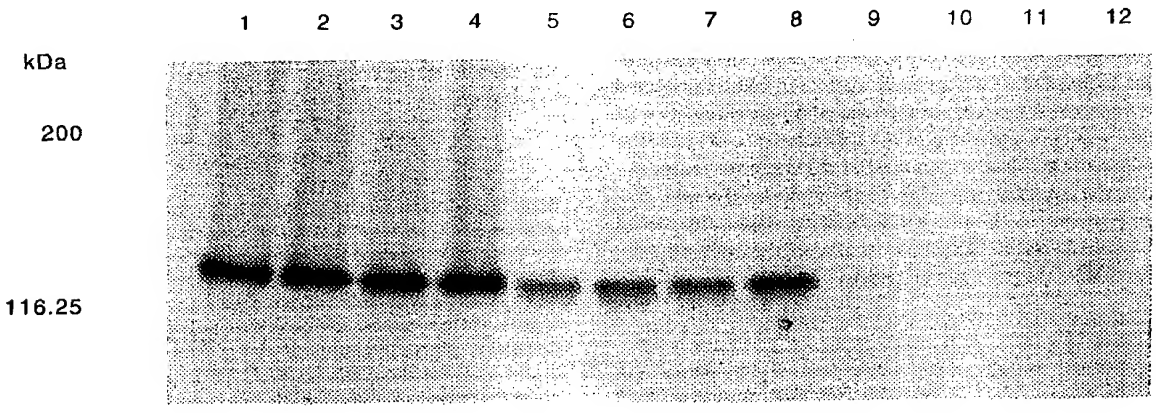

Fig. 1. An immunoblot showing the expression of hepatic low-density lipoprotein receptor (LDLR) in mice. C57BL/6J mice were fed either a low-fat diet (lanes 1–4) or a high-fat, high cholesterol diet (15% fat, 1% cholesterol, 0.5% sodium cholate) for 3 (lanes 5–8) or 18 weeks (lanes 9–13). Liver membrane proteins were solubilized, subjected to 3–13% SDS polyacrylamide gel electrophoresis and electrotransferred to nitrocellulose membranes. The LDR band was detected by using a polyclonal antibody against bovine adrenal LDLR and the enhanced chemiluminescence system.

ApoE-deficient mice was normal and comparable to triglyceride clearance in normal mice. However, over 60% of the injected emulsion CO remained in the plasma of ApoE-deficient mice 30 min after injection of double-labelled emulsions. The differences in clearance compared with control mice were statistically significant ($P < 0.05$ at 10 min and $P < 0.0001$ at 20 and 30 min), suggesting defective remnant clearance in mice deficient in ApoE. Consistent with the clearance data, over 60% of the injected [^{3}H]-CO was recovered in the livers of control mice, but only approximately 5% was recovered in the liver of ApoE-deficient mice, as shown in Fig. 2b. The hepatic uptake of [^{14}C]-TO was similar in control and ApoE-deficient mice (Fig. 2b). As a result of the accumulation of chylomicron remnants and probably other lipoproteins, mice deficient in ApoE develop atherosclerosis even when consuming a low-fat diet.[14] Clearance of

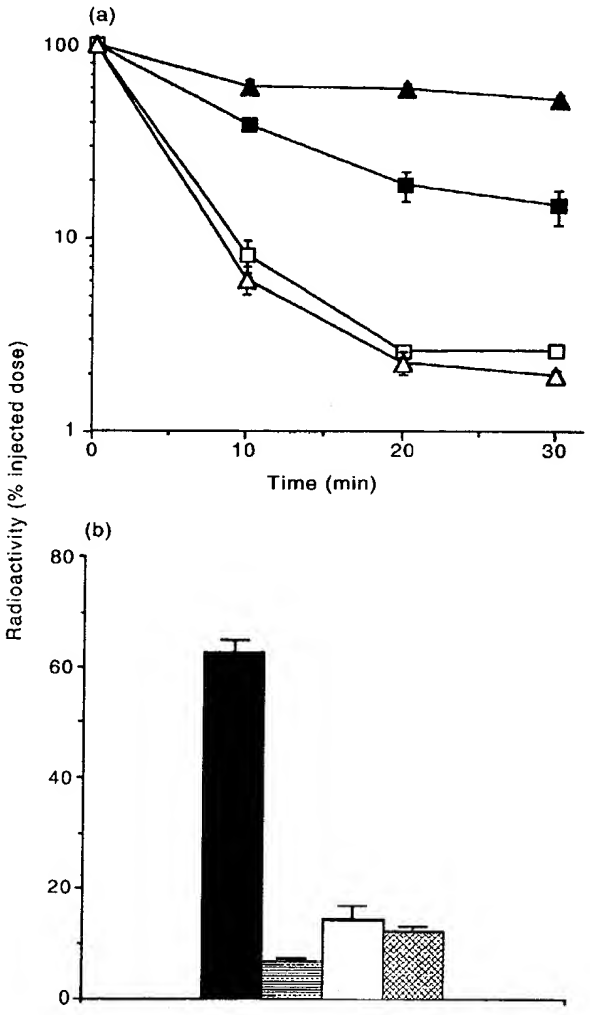

Fig. 2. Plasma clearance (a) and liver uptake (b) of injected chylomicron-like lipid emulsions in control C57BL/6J or apoE-deficient mice. (a) Emulsion lipids were labelled with [^{14}C]-triolein (TO) to trace the lipolysis and removal of emulsion triglycerides and with [^{3}H]-cholesteryl oleate (CO) to trace the clearance of the injected particles up to 30 min after injection. Results are the mean ± SEM with $n = 6$ for apoE-deficient mice and $n = 9$ for control mice. (■), CO clearance in control mice; (□), TO clearance in control mice; (▲), CO clearance in apoE-deficient mice; (△), TO clearance in apoE-deficient mice. (b) Radioactivity recovered in the liver of control C57BL/6J or apoE deficient mice. Mice were anaesthetized with avertin and animals were exsanguinated 30 min after the injection of radiolabelled emulsions. Results are the mean ± SEM with $n = 5$ for apoE deficient mice and $n = 15$ for control mice. (■), CO uptake in control mice; (□), TO uptake in control mice; ▨, CO uptake in apoE-deficient mice; (▨), TO uptake in apoE-deficient mice.

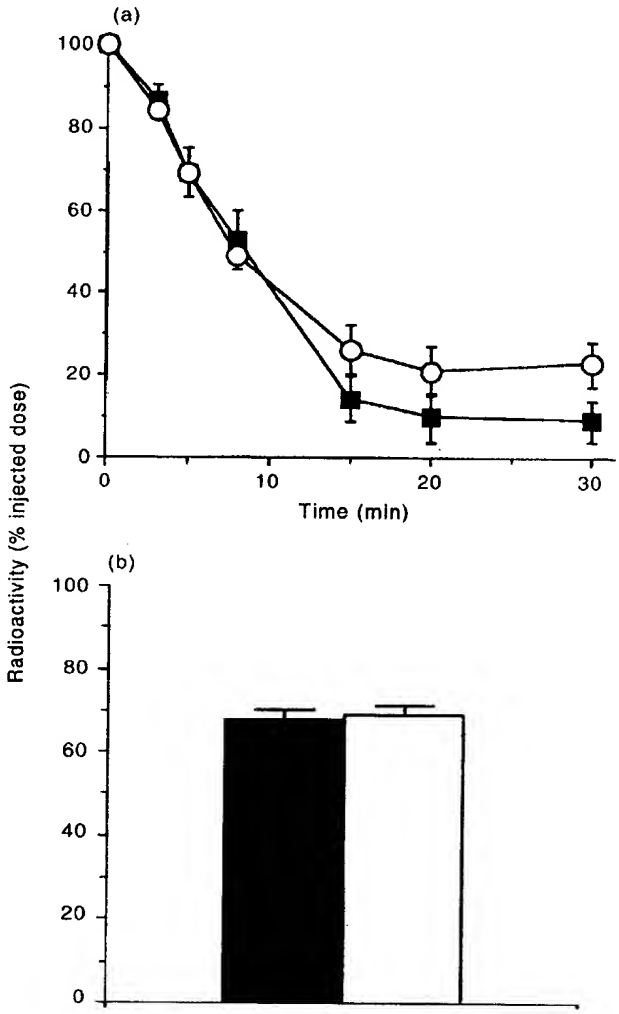

Fig. 3. Plasma clearance (a) and liver uptake (b) of injected lymph chylomicrons in control C57BL/6J or low-density lipoprotein receptor (LDLR)-deficient mice. Chylomicrons were labelled endogenously in the donor rats with [^{14}C]-palmitic acid and [^{3}H]-cholesterol. (a) The figure shows clearances of the [^{3}H]-label to trace the removal rate of chylomicron remnants up to 30 min after injection. Results are the mean ± SEM with $n = 6$ for each group. Three blood samples were taken from each animal with groups of mice used for time points 3, 5 and 8 min, and others for time points 15, 20 and 30 min. (■), CO clearance in control mice; (○), CO clearance in LDLR-deficient mice. Clearances of the [^{14}C]-label in control and receptor-deficient mice were similar. (b) Radioactivity recovered in the liver of control C57BL/6J, or LDLR-deficient mice. Mice were anaesthetized with avertin and animals were exsanguinated 30 min after the injection of radiolabelled chylomicrons. Results are the mean ± SEM with $n = 6$ for each group. (■), CO uptake in control mice; (□), CO uptake in LDLR-deficient mice.

lymph chylomicrons in ApoE-deficient mice was not feasible as the chylomicrons contained endogenous ApoE from the donor rats.

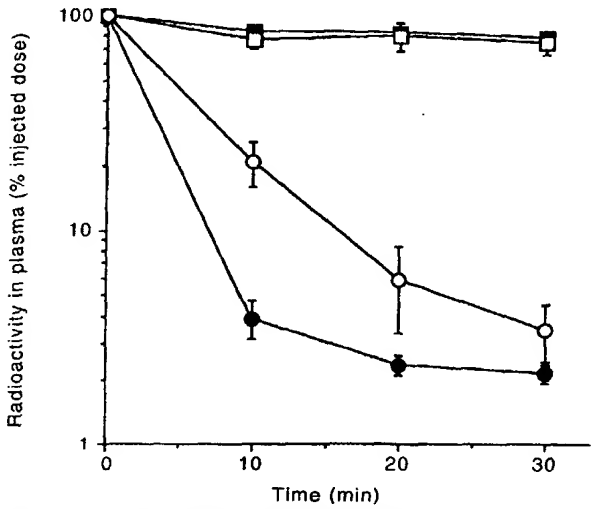

Fig. 4. The effect of heparin on plasma clearance of injected chylomicron-like lipid emulsions in low-density lipoprotein receptor (LDLR)-deficient mice consuming a high-fat diet. Emulsion lipids were labelled with [^{14}C]-triolein (TO) to trace the lipolysis and removal of emulsion triglycerides and [^{3}H]-cholesteryl oleate (CO) to trace the clearance of the injected particles up to 30 min after injection. Injection of heparin (5 U/animal) prior to the emulsion significantly increased the lipolysis of triglyceride, but had no effect on the clearance of emulsion remnant particles. (■), CO with heparin; (□), CO without heparin; (○), TO without heparin; (●), TO with heparin.

Chylomicron metabolism in gene knock-out mice deficient in the LDL receptor

We have shown recently that the clearance rates of chylomicron-like lipid emulsions were similar in LDL receptor-deficient and control mice.[2] Consistent with this finding, the plasma clearance rates of lymph chylomicrons in control and LDL receptor-deficient mice were also similar, as shown in Fig. 3a. Figure 3b shows that similar amounts of chylomicron remnants were recovered in the liver of the two strains of mice. There were no differences in the rate of lipolysis and the hepatic uptake of chylomicron triglycerides in control and receptor-deficient mice.

In LDL receptor-deficient mice, while chylomicron remnants were associated with the liver similarly as in control mice, internalization of remnants into hepatocytes was delayed. Confocal images of liver sections taken at time intervals after the injection of fluorescently labelled emulsions showed that remnants accumulated at the boundary of the sinusoidal spaces in LDL receptor-deficient mice, while remnants distributed evenly in the hepatocytes of the control mice from 5 to 20 min after injection.[2] At 3 h after the injection of the fluorescently labelled emulsions, no fluorescence was detected in the liver sections obtained from control mice, suggesting catabolism of remnants, whereas in mice deficient in LDL receptors, fluorescent remnants were evenly distributed in hepatocytes.[2]

The slow remnant catabolism was confirmed by the output of $^{14}CO_2$ in the expired breath of control compared with LDL receptor-deficient mice after injection of emulsions labelled with [^{14}C]-CO. Radioactivity in expired breath was significantly less in receptor-deficient mice compared with controls ($P < 0.0001$), indicating a defect or delay in the catabolism of remnants removed from the plasma.[11]

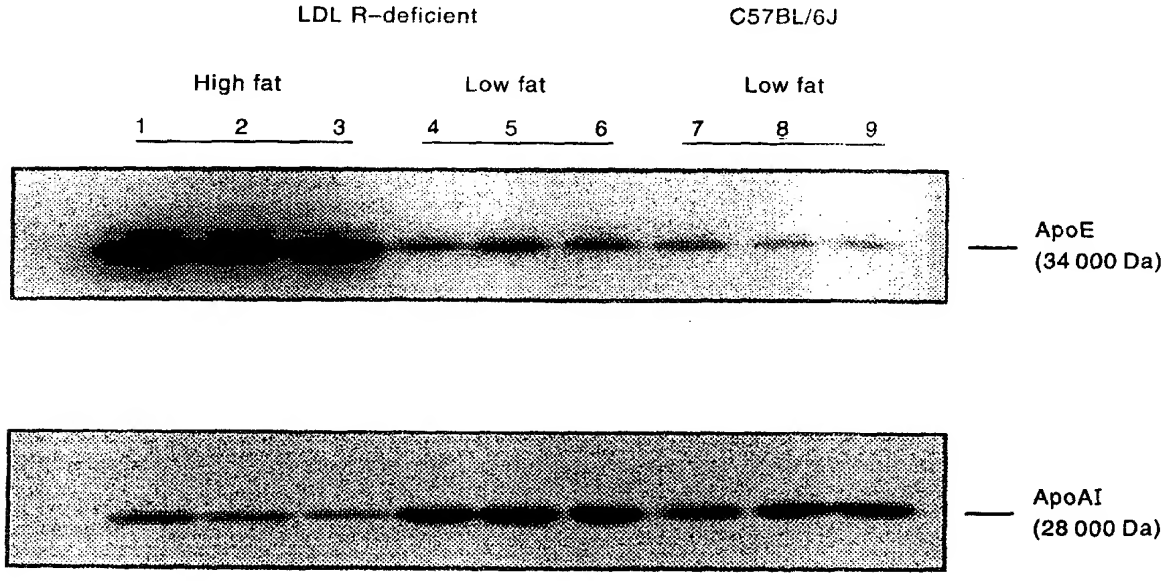

Fig. 5. An immunoblot showing the effect of a high-fat, high cholesterol diet on the plasma concentrations of apolipoprotein AI and E in low-density lipoprotein receptor (LDLR)-deficient mice. Mice (receptor-deficient and controls) were either fed a low-fat diet (lanes 4–6, 7–9) or a high-fat (receptor-deficient only) for 3 weeks (lanes 1–3). Plasma proteins were subjected to 5–25% SDS polyacrylamide gel electrophoresis and electrotransferred to nitrocellulose membranes. The apolipoprotein bands were detected by using polyclonal antibodies against the apoAI and apoE, respectively, and the enhanced chemiluminescence system.

Our results suggest that under normal circumstances, chylomicron remnants are rapidly internalized by LDL receptors and are catabolized in hepatocytes. When the receptor is absent, remnants are taken up by the liver via a second ApoE-dependent pathway, first to the sinusoidal space, with subsequent slow endocytosis and catabolism. Nevertheless, the alternative pathway of hepatic remnant uptake is sufficient to prevent LDL receptor-deficient mice from accumulating B_{48}-containing lipoproteins in the circulation. In an attempt to isolate the mechanism of this pathway, we performed competitive studies of emulsion clearance. Our results show that reagents, including lactoferrin, heparinase and suramin, which inhibit the functions of heparan sulfate all retarded remnant clearance, while heparin accelerated remnant clearance.[2]

Mice deficient in LDL receptors develop atherosclerosis only after consuming a high-fat, high cholesterol diet.[15] This is consistent with our findings that plasma clearance and hepatic uptake of chylomicron remnants were severely retarded in receptor-deficient mice after consuming a high-fat (15% fat, 1% cholesterol, 0.5% sodium cholate) diet for 3 weeks. Injection of heparin accelerated lipolysis of emulsion triglyceride but had no effect on plasma removal or liver uptake of chylomicron remnants as shown in Fig. 4. The high-fat diet also promoted three- and five-fold increases in plasma triglyceride and cholesterol, respectively, and a decrease in HDL-cholesterol. As shown in the immunoblots presented in Fig. 5 and consistent with these results, plasma ApoAI (lanes 1–3, lower panel) decreased, while ApoE (lanes 1–3, upper panel) increased significantly in receptor knock-out mice compared with knock-out mice consuming a low-fat diet (lanes 4–6) or control mice consuming a low-fat diet (lanes 7–9).

Antisense gene inhibition in HepG2 cells

Results from *in vivo* competition studies suggest the involvement of heparan sulfate proteoglycan in the uptake of remnants by liver cells in both the control and LDL receptor-deficient mice. To further investigate the role of heparan sulfate proteoglycan in remnant uptake, we evaluated the effect on remnant uptake as a result of reduced proteoglycan expression by antisense gene inhibition of syndecan, the main core protein of liver proteoglycans, in HepG2 cells.

The cDNA of human syndecan-1 was used as a template to prepare antisense ODN and phosphorothioate ODN. HepG2 cells were transfected with various antisense and sense ODN mediated by the cationic liposome DOTAP 24 or 48 h before remnant uptake studies. A synthetic antisense oligonucleotide with the sequence 5'-GCGGG-TTCCGCTGCTCGATG-3', which is complementary to a 20 nucleotide sequence upstream of the initiation site of the syndecan gene, was found to suppress HSPG expression. Incorporation of [^{35}S] by cells radiolabelled for 48 h with [^{35}S]-sodium sulfate (carrier-free) in the presence of the antisense oligonucleotide was reduced by approximately 25% compared with controls (B-C Mortimer *et al.*, unpubl. obs., 1997). Remnant binding decreased by approximately 50–70% in cells incubated with the antisense oligonucleotide compared with control cells incubated with a sense ODN or with DOTAP only (B-C Mortimer *et al.*, unpubl. obs., 1997).

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